A PHYSICO-CHEMICAL STUDY OF CRACKER DOUGH FERMENTATION

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, 1924

By

ARNOLD H. JOHNSON, M.S.

JUNE, 1924

3781
M66J
UNCLASSED
STANFORD UNIVERSITY LIBRARIES
WITHDRAWN

A PHYSICO-CHEMICAL STUDY OF CRACKER DOUGH FERMENTATION

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, 1924

By

ARNOLD H. JOHNSON, M.S.

JUNE, 1924

CONTENTS

	Page
Introduction	3
Problem	4
Experimental	5
A survey of the crackers produced in the industry	5
A survey of the flours used in the manufacture of crackers	10
Viscosity studies	17
Changes in hydrogen-ion concentration which occur during the fermentation process and subsequent baking of the dough into crackers	18
Changes in hydrogen-ion concentration which take place in the cracker during the baking process	27
Chemical control of the neutralization of the acids produced during fermentation	30
Proteolysis in cracker dough fermentation	47
Effect of hydrogen-ion concentration on the dispersion of flour proteins	53
Differentiation of changes produced by proteolysis from those produced by hydrogen-ion concentration	57
Effect of prolonged fermentation on viscosity	61
Diastatic activity in cracker doughs	64
Losses in a cracker dough due to fermentation	67
Other processes of cracker manufacture	69
Attempts to increase the yield of crackers	70
Discussion	73
Summary and conclusions	78
Literature cited	81
Acknowledgements	83
Biography	86

A PHYSICO-CHEMICAL STUDY OF CRACKER DOUGH FERMENTATION[1]

By Arnold H. Johnson

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station, St. Paul, Minn.

The earliest form of bread produced by primitive man was probably a sort of biscuit baked on a heated stone. In the Orient and other parts of the world, certain races still produce such a biscuit or flat bread as an important part of their bread ration. It is a far cry from the simple, unleavened baked dough of the ancients to the modern soda biscuit, however. Advances in methods of flour production and contributions of bakers and manufacturing chemists have effected marked improvement in the palatability and other desirable properties of this important food. The manufacturing process has become so highly developed, particularly in the matter of mechanical equipment, as to result in the production of most of the crackers of commerce in large plants. This situation facilitates the application of improvements in this industrial field and makes possible the use of any suitable control measures which may be developed.

As flour is the basic raw material of cracker dough, it follows that the chemistry of flour strength becomes of immediate concern. During the last quarter century extensive researches have been conducted by numerous chemists with a view toward determining and measuring those components and characteristics of flour which influence its strength and bread-making qualities. The literature of various phases of this subject has been reviewed recently by Rumsey (1922), and Sharp and Gortner (1923) and need not be repeated here. Almost every chemical constituent, and every measurable physical and physico-chemical property of flour has been studied, in order to ascertain the effect of each upon the "resultant of forces" expressed in the baked product. Since the colloid chemist has entered the field, the tendency has

[1] A paper based on this thesis has appeared in Cereal Chemistry, Vol. I, No. 7, Dec. 1924.

been toward stressing the significance of the colloidal properties of the complex material known as dough. Enzyme phenomena have not been overlooked, however, and as in both bread and cracker production we find ourselves dealing with a living plant organism, yeast, which functions through the activity of its several enzymes, it becomes necessary to consider the metabolism of this fungus. In organizing the research which resulted in the preparation of this paper, consideration was given to the possible effect of the variables which determine the course of cracker dough fermentation and properties of the finished product.

Problem

Before undertaking a study of the problems involved in the cracker sponge and dough fermentation process, an effort was made to determine the desirable physical and chemical properties of crackers. Crackers were collected from factories throughout the United States, and were subjected to the determination of hydrogen-ion concentration, residual carbonates, and development of rancidity on storage.

At the same time flours used in the cracker industry were collected from the same factories and subjected to the determination of ash, crude protein, viscosity of acidulated suspensions of flour in water, and diastatic activity.

Hydrogen-ion concentration of the cracker sponge and dough was determined at various intervals during the process of fermentation of cracker sponges and doughs, which determinations served to indicate the rate of fermentation, whether the proper quantity of sodium bicarbonate had been added to the dough, and the colloidal and physical state of the proteins of the dough at that particular time, in so far as these are related to hydrogen-ion concentration.

Hydrogen-ion concentration of the finished crackers was determined. This determination also indicated whether the proper quantity of sodium bicarbonate had been added to the dough in each case, and added to our information concerning the chemical changes occurring in the cracker during the baking process.

Titratable acidity of the sponge was determined as an index of the proper quantity of sodium bicarbonate to add to the dough.

The effects of the extended fermentation period on the chemical properties of the proteins were determined, so far as these effects are indicated by the protein fraction soluble in water, the fraction non-precipitable with copper hydroxide, and that non-precipitable with stannous chloride. These data served to indicate whether the proteins had undergone hydrolytic cleavage.

The physico-chemical properties of the proteins, including the viscosity of the acidulated dough suspensions, were noted and progressive

changes in the quality constants of the glutenin were determined at various intervals during the fermentation period. Changes in the latter served as a means of detecting modifications in the gluten which were difficult to determine by chemical methods.

The physical properties of the dough resulting from extended fermentation were observed to the extent that these could be detected by the Chopin extensimeter, which indicated any changes occurring in the elasticity and tenacity of the dough. The concentration of reducing sugars in the dough at various stages of the fermentation process was determined, as such data indicated the relative rates of fermentation and diastatic activity.

Carbon dioxide formed during fermentation was determined in one instance, this affording a measure of the loss in dry matter which occurred during the fermentation process.

The changes occurring in a no-sponge-time process were noted and compared with the changes effected in the usual fermentation practice.

Experimental

A survey of the crackers produced in the industry.—Jessen-Hansen (1911), and Cohn and Henderson (1918) indicated the importance of hydrogen-ion concentration as applied to bread-making. Bailey and Sherwood (1923) made further use of the hydrogen electrode in determining the march of hydrogen-ion concentration in the course of the fermentation of a bread dough. From the significance of such measurements in the bread industry it appeared that determinations of the hydrogen-ion concentration of cracker extracts might be an index to the properties of the best crackers. Such information should be especially important in the light of our knowledge of the effects of the hydrogen-ion concentration on physical and colloidal properties of the proteins of the flour.

The apparatus used for the determination of the hydrogen-ion concentration was essentially that used in the potentiometric method described by Michaelis (1911), and included a potentiometer, a standardized Weston cell as the source of constant reference potential, a tenth-normal calomel half-cell, a Bailey (1920) hydrogen electrode, and a galvanometer as the null point instrument. The solutions used in the determinations were prepared in the following manner: The crackers were ground in a mortar until a fine meal was obtained. To 15 grams of this meal in an Erlenmeyer flask was added 100 cc. of distilled water and the mixture was shaken until a smooth suspension was obtained. After allowing the suspension to stand for 10 minutes, it was poured into a centrifuge tube and the solid material centrifuged out. The hydrogen-ion concentration of the clear liquid was then determined.

The concentration of cracker meal, 15 grams per 100 cc. of water, was chosen because at this concentration slight errors in weighing the material made little, if any, difference in the accuracy of the determination. The data in Table I indicate the small effect of concentration on the pH of the solution, through the range from 10 to 20 grams of cracker meal per 100 cc. of water.

TABLE I

RELATION OF CONCENTRATION OF CRACKER MEAL TO HYDROGEN-ION CONCENTRATION OF THE EXTRACT

Grams of cracker meal per 100 cc. of water	pH
5	7.84
10	8.10
15	8.06
20	8.08

In addition to the potentiometric determinations of hydrogen-ion concentration, colorometric determinations were made. Methyl orange, methyl red, phenol red, alizarine red, brom cresol purple, brom cresol blue, thymol blue, and phenolphthalein were used as indicators. Observations made by the colorometric method agreed with the more accurate determinations by the potentiometric method, within the limits of accuracy anticipated in such determinations.

In this preliminary work it was noted that the more alkaline cracker extracts were characterized by a greenish-brown color not possessed by less alkaline extracts. The order of brownness was the order of their pH, the brownest extract having the highest pH or greatest alkalinity. That this brownness was due to the effects of the excess sodium bicarbonate will be shown in another section.

At the outset it seemed that the quantity of residual carbonate in the cracker might be an important factor in considering the properties of the cracker, and such determinations were accordingly made, using Truog's method (1915). Twenty-five gram samples of the cracker meal were acidified and the carbon dioxide was aspirated and absorbed by barium hydroxide solution in a modified Truog tower. While the quantity of excess sodium bicarbonate in the dough is of extreme importance, its determination in the baked cracker yielded no significant results except to indicate that carbonates are present in the baked cracker in extremely small quantities.

One of the problems of the cracker industry, somewhat outside the scope of this research, to be sure, is rancidity. It was a common belief that acid crackers became rancid and for this reason alone crackers on the alkaline side of neutrality were more desirable. These cracker samples were accordingly stored and the development of rancidity was noted, the purpose being to correlate, if possible, rancidity with reaction

or with type of shortening or some other determinable factor. The data in Table II show considerable variation in hydrogen-ion concentration of crackers from different factories, the most alkaline having a pH of 8.81 and the most acid having a pH of 5.73. Of 60 samples, 75 per cent gave extracts which were on the alkaline side of neutrality while the remaining 25 per cent were on the acid side. The average pH for the whole series was 7.84, being distinctly on the alkaline side of neutrality. During a storage period of about seven weeks, the average pH fell from 7.84 to 7.50 indicating a tendency toward increasing acidity with the lapse of time. Some of the individual samples changed at a rapid rate, others changed slowly or not at all. A few were more alkaline after storage. In view of the general tendency in the opposite direction, any observed increase in alkalinity was no doubt due to errors in sampling. Attempts to correlate the development of rancidity with type of shortening or the reaction of the crackers were futile. The percentage of alkaline crackers which became rancid or very rancid was about the same as the percentage of acid crackers. Thus there were 45 samples of alkaline crackers, of which 14 became rancid. There were 15 samples of acid crackers, of which 4 became rancid. This evidence indicates, if anything, that from the standpoint of developing rancidity, the acid cracker may be slightly more desirable, altho such a conclusion is hardly justified in view of the few data available.

Of the 29 samples more alkaline than the average of pH 7.84, 9 were classified as becoming rancid, while of the 31 samples on the acid side of pH 7.84, 10 showed rancid properties after storage. These data indicate that there was no correlation between the hydrogen-ion concentration of crackers and the development of rancidity in such crackers.

TABLE II

RESIDUAL SODIUM BICARBONATE, pH VALUES BEFORE AND AFTER STORAGE, TYPE OF SHORTENING USED, AND RANCIDITY OBSERVATIONS ON SERIES OF CRACKERS COLLECTED FROM FACTORIES THROUGHOUT THE UNITED STATES

Sample No.	$NaHCO_3$ Per cent	pH		Type of shortening	Quantity of shortening used per bbl. flour Lbs.	Rancidity Observations on stored crackers	
		April 26	June 15			Odor	Taste
42	.0236	8.81	8.66	Lard	18	Fair	Good
3	.0192	8.79	8.72	Lard	23	Very rancid	Not tasted
1		8.74	8.70	Lard	20	Rancid	Not tasted
13		8.71	8.61	Hcs*	24	Sweet	Good
36		8.69	8.74	Lard	16	Sweet	Good
37		8.66	8.50	Corn oil and beef fat	18	Rancid	Not tasted
43	.0256	8.55	8.42	Lard	17	Rancid	Not tasted
31	.0230	8.45	8.42	Lard and Florolene	20	Sweet	Good
33	.0256	8.40	8.55	Lard	20	Rancid	Not tasted
52	.0231	8.37	8.61	———	—	Rancid	Not tasted
40	.0153	8.37	8.13	Cotton oil and compound	22	Very rancid	Not tasted
2	.0218	8.35	8.28	Oleo	14.3	Sweet	Good
7		8.28	8.32	Lard	22	Fair	Off
14	.0205	8.27	7.79	Hcs	22	Sweet	Good
51	.0230	8.23	8.12	Hcs	21.8	Rancid	Not tasted
39	.0205	8.20	7.57	Lard	15	Rancid	Not tasted
34	.0102	8.18	7.84	Lard	16	Sweet	Good
28	.0256	8.18	8.23	Lard	17	Fair	Off
45	.0128	8.15	8.18	Lard	12	Fair	Off
49	.0205	8.13	8.45	———	—	Fair	Off
17	.0205	8.06	7.93	Lard	18	Fair	Good
60a	.0179	8.06	7.91	———	—	———	———
26	.0179	8.05	7.89	Lard	9.25	Sweet	Good
56	.0256	8.01	7.64	Lard	—	Sweet	Good
46	.0179	8.01	8.45	Lard	15	Sweet	Good
24	.0205	8.00	7.89	Lard	20	Sweet	Good
48	.0282	7.93	7.79	———	—	———	———
27	.0179	7.90	7.85	Hcs	15	Sweet	Good
15		7.81	6.93	Lard	18	Sweet	Good
18	.0205	7.67	7.61	Hcs	21.8	Rancid	Not tasted

TABLE II—*Continued*

Sample No.	$NaHCO_3$ Per cent	pH April 26	pH June 15	Type of shortening	Quantity of shortening used per bbl. flour Lbs.	Rancidity Observations on stored crackers Odor	Taste
11	.0154	7.61	7.25	Lard	15	Rancid	Not tasted
19	.0154	7.59	7.52	Hcs	20	Sweet	Good
58		7.57	....	———	—	———	———
5	.0128	7.56	7.51	Corn oil	20	Very rancid	Not tasted
6	.0128	7.51	7.31	Lard	16	Fair	Good
35		7.44	7.39	Corn oil	16	Very rancid	Not tasted
50	.0102	7.40	8.08	Lard	20	Fair	Off
30	.0102	7.39	7.32	Lard	18	Very rancid	Not tasted
44	.0128	7.39	7.32	Lard	18	Rancid	Not tasted
59		7.34	7.24	Lard	16	Sweet	Good
55		7.27	....	Lard and oleo	22	Fair	Off
25		7.18	7.13	———	—	Fair	Off
12	.0076	7.17	7.15	Lard	20	Fair	Good
8	.0077	7.08	7.02	Oleo and lard	18	Sweet	Good
29	.0000	7.00	6.94	Lard	15	Very rancid	Not tasted
38	.0000	6.85	6.77	Lard	—	Sweet	Good
21		6.81	6.75	Hcs	18	Very rancid	Not tasted
20		6.78	6.73	Lard	16	Sweet	Good
32	.0128	6.76	6.76	Lard	22	Rancid	Not tasted
9		6.76	6.74	Lard	12	Sweet	Good
16	.0000	6.63	6.54	Lard	20	Rancid	Not tasted
22	.0000	6.59	6.54	Lard	18	Fair	Good
47	.0051	6.53	6.43	Oleo	13	Sweet	Good
4	.0000	6.51	6.46	Oleo	18	Sweet	Good
41		6.32	6.26	Lard and Hcs	14 to 20	Fair	Off
23	.0051	6.29	6.27	Lard	17	Fair	Off
10	.0000	6.22	6.20	Lard	—	Fair	Off
57		6.15	6.10	Lard	10	Fair	Off
53	.0038	6.07	5.92	———	—	Fair	Good
54	.0051	5.71	5.73	———	—	Fair	Off
	Average	7.84	7.50				

* Hcs = all-hydrogenated cottonseed oil.

Survey of the flours used in the manufacture of crackers.—It is generally known that the flours which are commonly used in bread-making differ chemically and physically from those which are used in the manufacture of crackers. The bread baker requires a strong flour with a relatively high percentage of gluten. The cracker baker, on the other hand, prefers a weak flour with a smaller percentage of gluten. The bread flours for the most part are milled from hard wheats produced in regions where the rainfall is light, that is, such regions as the Red River valley, the Dakotas, Montana, Nebraska, and Kansas. The cracker flours are usually milled from soft winter wheats produced in regions of heavier rainfall, or in districts where the wheat can be irrigated. Leighty and Martin (1922) give the following acreage of soft red winter wheats in the ten leading soft red winter wheat states.

	Acres
Missouri	3,461,700
Indiana	2,354,500
Ohio	2,250,800
Illinois	2,153,400
Texas	1,488,500
Kansas	1,424,300
Pennsylvania	1,172,300
Oklahoma	1,056,700
Virginia	887,400
Kentucky	737,300
Other	3,704,500

According to the data of Shollenberger and Clark (1924) such wheats have an average crude protein content of 11.3 per cent as compared with 12.6 and 13.6 per cent in the average hard red winter and hard red spring wheats, respectively.

Questionnaires were sent to cracker factories throughout the United States and Canada seeking data concerning the origin and characteristics of the flours used in the industry. Each factory was also asked to send in samples of the various flours used. These data as well as the results of chemical analysis are presented in Table III. The flours were divided into groups according to their origin in order that common properties of each group, if such exist, may be more evident. Thirty-four samples were of unknown origin. Twenty-three flours were milled from Missouri wheat, 12 from Indiana wheat, 10 from Ohio wheat, and 9 from Illinois wheat. This is the order of production of soft red winter wheat in these states. Sixteen of the flours were milled from Pacific Coast wheats and 8 from Michigan wheats, while a few were milled from Idaho and Canadian wheats. The wheats used in milling these flours originated in regions which produce typical biscuit flour wheats, altho certain flour samples in this series might be classified as bread flours rather than typical cracker flours.

The grade of the flours as reported in the questionnaire is given in Table III. The ash content of these flours was determined, as this is a common method for estimating the grade or degree of extraction of flour. The lower grades of flour have a higher ash content than the higher grades, owing to the presence in low-grade flours of the branny material, which contains a higher percentage of inorganic salts. The lower, or clear flours, are accordingly darker in color, and for that reason are not so desirable for certain purposes. Stockham (1920), and Johnson (1923) have shown that doughs made with clear flours ferment at a much more rapid rate than those made with higher grade, or patent flour. This may possibly be ascribed in part to the presence of phosphates in these flours. Harden and Young (1908) have shown the accelerating effect of phosphates on carbon dioxide production. Hence when such flours are mixed into doughs and allowed to ferment, the grade of flour, on account of its phosphate content, may have a significant effect on the rate of fermentation. The higher diastatic activity of flours of the lower grades may also so operate as to cause fermentation to be more rapid.

The data in Table III and Figure 1 indicate that the flours examined ranged in ash content from .35 to 1.71 per cent, the average being .49 per cent. The greater percentage of the flours, however, so group themselves that the ash content lies between .40 and .56 per cent. This means that about 80 per cent of these flours which were used in the cracker industry would be classified probably as "long patents" or "straights" in the flour trade. In Figure 1 this tendency of the flours so to group themselves as to be identified as belonging to the "long patent" or "straight" grades is easily noted. Four special purpose flours having ash contents of .82, .95, .99, and 1.71 per cent are not shown in Figure 1.

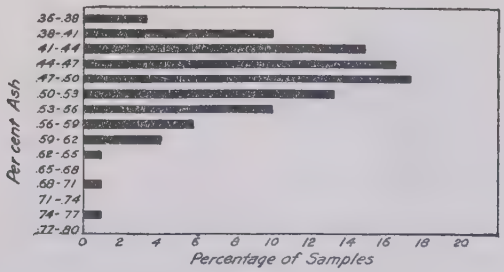

Fig. 1. Distribution of Flours Grouped According to Ash Content

The length of each line indicates the percentage of the total number of samples falling in the group. The graph was prepared from data which resulted from the study of the 121 flours obtained from the stocks of biscuit and cracker manufacturers throughout the United States and Canada.

That the protein content of flour is an important factor in flour strength is generally recognized, and protein determinations are commonly made in control laboratories. The work of Snyder (1899), Shutt (1907), Bailey (1913), and Thomas (1917), shows that, on the average, the bread-making flours contain a higher percentage of total protein than the soft wheat flours commonly used in biscuit making. Zinn (1923) points out that while this correlation between bread-making strength and protein content is significant and positive it is by no means perfect. Hence, for the most part we should expect to find the flours of high protein content more suitable for bread than the low protein flours. Medium protein flours, on the other hand, appear more suitable for biscuit making, while low protein flours are conveniently used in certain types of pastry.

In order to ascertain the suitability of the flours used in the cracker industry for the uses to which they were put, the percentage of protein of the same series of flours was determined following the method of the A. O. A. C. (1919), and the data are presented in Table III. These data show no significant differences in the protein content in the flours milled from wheats grown in different sections of the country, with the exception perhaps that certain of the Washington and Oregon flours were higher in protein. The protein content of the flour milled from California wheat was remarkably low, but as only one such flour was examined, no generalization can be made. Shaw and Gaumnitz (1911) state, however, that a low protein content is characteristic of California wheat. In most of the cases in which relatively high protein flours were encountered it was believed that this was due to the lower grade of the flour. This view is based on a consideration of the ash content of those particular flours. There are a few flours, however, which appear to have the properties usually ascribed to the stronger flours.

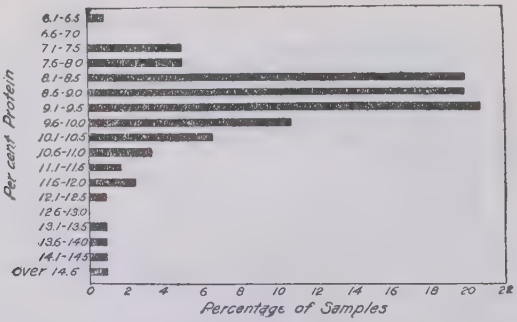

Fig. 2. Distribution of Flours Grouped According to Crude Protein Content

The length of each line indicates the percentage of the total number of samples falling in the group. The graph was prepared from data which resulted from the study of the 121 flours obtained from the stocks of biscuit and cracker manufacturers throughout the United States and Canada.

Thus flour No. 55, milled from Missouri wheat, has a crude protein content more than 2 per cent higher than the average for flours of this ash content. The distribution of the flours in groups based upon protein content is shown in Figure 2. Seventy per cent of the flours were in the range between 8.0 and 10.0 per cent of protein, and of this number approximately 40 per cent lie between the limits of 8.0 and 9.0 per cent of protein. The average protein content of the 121 flours studied was 9.28 per cent, which is at least 2 per cent lower than is desired in bread flours at the present time.

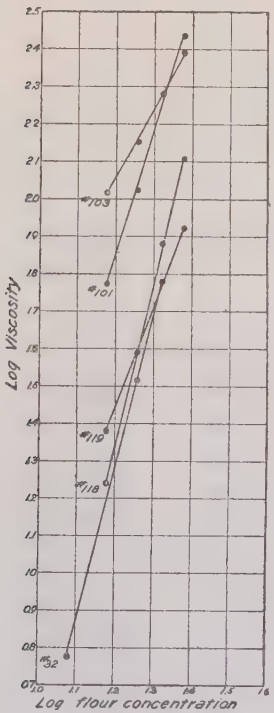

Fig. 3. Logarithmic Viscosity —Concentration Curves of Several Flours Differing in Gluten Quality.

It is the general opinion of men engaged in cracker manufacture that flours of different grade and protein content are used for the production of various types of biscuits. For example, the flour used in the soft sponge stage of cracker manufacture is believed to be stronger than that added to the sponge at the time the dough is prepared. Flours used in various types of cakes are thought to be of relatively lower strength than those used in doughs which are subjected to fermentation with yeast.

In order to determine whether this opinion was in accordance with the facts, so far as could be determined by ordinary chemical analysis, the cracker manufacturers who submitted samples of flour were asked to specify the purposes for which the flours were to be used. These data are also given in Table III and, as will be noted, most of the flours were used for several purposes, a few being used in all five types of goods listed in the table. The asterisks in the table indicate the purposes for which the flours were used.

A study of this table will show that 68 of the flours were used in cracker sponges. These flours had an average ash and protein content of .50 and 9.59 per cent, respectively. Sixty-five flours were used in the preparation of the dough for cracker manufacture. These flours had ash and protein contents of .50 and 9.45 per cent, respectively. There certainly appeared to be no significant differences in either grade of flour or protein content of the flours used in cracker sponge and

cracker dough in so far as is indicated by the averages calculated from the data in Table III.

TABLE III

DATA ON THE FLOURS USED IN THE CRACKER INDUSTRY
Origin, Grade, Ash Content, Protein Content, Quality Constant *b*, and Types of Goods in which Flours were Used

No.	Grade	Ash	Protein	Quality Constant (b)	Types of goods in which the flours were used* Sponge	Dough	Lunch milk type	Cutting machine cakes	Drop-cake machine type
			Wheat of unknown origin						
		%	%	%					
1	Clear	.57	7.92	3.15		*			
2	Patent	.44	9.80	3.81	*	*		*	*
3	"	.42	8.15	3.02	*	*		*	*
4	Clear	.52	9.41	3.35		*			
5	Patent	.40	8.38	3.49	*	*		*	*
13	"	.56	9.29	3.14	*		*		
14	Cut off	.53	8.95	4.03	*	*		*	
15	Patent	.56	10.15	4.22		*	*	*	*
16	"	.42	8.04	4.19				*	*
17	"	.61	10.09	2.75				*	*
18	Cut off	.74	10.26	3.19				*	*
19	Clear	.95	10.15	3.50				*	*
31	———	.43	8.55	3.39		*			
34	———	.51	8.72	3.76	*				
40	Patent	.46	7.52	3.89				*	*
45	"	.42	8.78	3.37				*	*
47	"	.49	9.35				*		
54	"	.44	9.12		*	*		*	*
61	———	.52	8.72		*				
73	Patent	.59	9.69		*			*	
74	Straight	.50	9.18		*	*		*	
75	"	.40	8.66		*	*		*	
76	Patent	.54	11.00	3.42	*			*	
77	"	.37	8.61		*	*		*	*
78	Straight	.46	9.23		*	*		*	*
90	Clear	.45	8.09			*		*	
91	Patent	.47	8.26			*	*	*	
92	Clear	.49	9.29			*		*	
93	Patent	.38	8.44	3.77			*		
94	"	.36	9.12		*	*			
95	Straight	.46	9.63		*				
99	———	.44	8.49		*			*	*
100	———	.44	8.89		*	*		*	*
115	Patent	.35	8.89						
			Milled from Missouri wheat						
20	Patent	.43	9.52	3.31	*				
21	Straight	.60	9.23	4.17				*	*
22	Patent	.99	9.80	2.87	*				
23	"	.48	9.12	3.14	*				
24	"	.45	8.49	3.44					
25	"	.40	8.84	3.19	*		*	*	*
26	"	.43	9.46	3.19		*			*
38	Straight	.42	9.75	2.83	*			*	*
39	Patent	.40	9.12	3.18	*				
41	"	.42	9.29	3.32	*	*		*	
51	"	.49	9.63		*				
55	"	.41	11.29	3.13	*	*		*	*
69	"	.40	7.87		*		*	*	*

*The asterisks indicate the types of goods in which the particular flours were used.

TABLE III—*Continued*

No.	Grade	Ash	Protein	Quality Constant (b)	Types of goods in which the flours were used[a]: Sponge	Dough	Lunch milk type	Cutting machine cakes	Drop-cake machine type
83	Straight	.53	9.46		*	*	*	*	*
84	Patent	.38	8.55		*	*			
85	Straight	.46	10.66		*			*	*
87	Patent	.37	8.61					*	*
88	Straight	.52	10.03		*	*		*	
89	"	.53	9.75			*		*	
111	Patent	.44	9.58		*	*			
112	Straight	.50	9.80		*	*			
113	"	.47	10.09		*	*			
114	"	.43	10.09					*	*
			Milled from Indiana wheat						
9	Patent	.48	8.95	3.73	*	*			
10	"	.43	9.46	3.22	*	*			
27	"	.49	8.66	3.05		*			
28	"	.50	8.38	4.34	*				
37	"	.44	9.01	3.59	*			*	*
58	"	.52	8.66			*		*	*
67	Straight	.46	9.58			*		*	
68	Patent	.42	8.89			*	*	*	*
96	Straight	.38	7.24	3.55			*	*	*
97	"	.55	8.38		*		*	*	
107	"	.58	9.41		*	*			
109	Patent	.40	9.18		*			*	*
			Milled from Ohio wheat						
6	Patent	.40	8.38	3.15	*				
11	Straight	.41	8.21	3.22	*	*	*	*	
12	Clear	1.71	13.11	2.57	*	*	*	*	*
60	Straight	.49	8.49			*			*
62	Patent	.43	8.15		*		*		*
63	Straight	.50	8.44			*	*		*
71	Patent	.46	9.41			*			
72	Straight	.50	8.72			*	*		*
79	Patent	.51	8.95		*				
110	"	.44	9.69						
			Milled from Oregon and Washington wheat						
8	Straight	.51	8.61	4.00			*	*	*
35	Patent	.39	10.60	2.57	*				
36	"	.48	7.87	3.36					
49	Straight	.46	11.74	2.77		*	*	*	
50	Patent	.49	11.63	2.35	*		*	*	
52	"	.49	7.47						*
64	Straight	.54	8.49					*	*
65	Cut off	.82	15.22	2.03	*	*			
66	Straight	.52	14.31	2.27	*	*			
101	Patent	.56	10.72	3.36		*	*	*	
102	Straight	.63	12.43	3.13	*	*			
103	"	.56	13.79	1.85	*	*			
104	Clear	.60	11.46	3.67	*	*			
106	Patent	.50	7.18	3.27			*	*	*
121	Straight	.55	11.63					*	*
			Milled from California wheat						
105	Patent	.48	6.33	3.57			*	*	*
			Milled from Illinois wheat						
7	Straight	.51	8.44	3.92		*		*	
53	"	.49	8.66			*			

TABLE III—*Continued*

No.	Grade	Ash	Protein	Quality Constant (b)	Types of goods in which the flours were used* Sponge	Dough	Lunch milk type	Cutting machine cakes	Drop-cake machine type
59	Patent	.49	8.72	3.34	*			*	*
70	"	.42	8.21			*	*	*	*
80	Straight	.53	9.29	3.06	*	*		*	*
81	Patent	.45	7.98			*		*	*
82	Straight	.47	8.78		*	*		*	*
86	"	.48	9.23			*		*	*
108	"	.53	9.23		*	*			
			Milled from Michigan wheat						
29	Patent	.41	7.92	3.57			*	*	*
30	"	.53	8.38	3.53			*	*	*
32	———	.56	7.87	4.09		*		*	
33	———	.42	8.21	3.87		*		*	*
56	Patent	.41	8.21			*		*	*
57	Straight	.44	10.26			*		*	*
98	"	.47	8.95		*			*	
116	"	.52	10.03						
			Milled from Idaho wheat						
42	Patent	.44	8.21	3.31	*	*	*		*
43	Straight	.53	8.61	2.97	*	*		*	
44	Clear	.60	10.15	2.91	*			*	
46	Patent	.47	9.18	3.33	*	*	*	*	*
48	Straight	.48	9.35		*				
			Milled from Ontario, Canada, wheat						
117	———	.45	10.20	2.91	*				
118	———	.48	9.63	4.43	*		*		*
119	———	.53	8.84	2.66	*	*	*	*	*
120	———	.46	8.26	3.01	*	*	*	*	*
Number of flours used for each type of goods.....					68	65	30	71	58

Thirty of the flours were used in the lunch milk type of biscuit. These flours had an average ash content of .51 per cent and an average protein content of 8.92 per cent. Seventy-one flours were used in the manufacture of cutting machine cakes, and 58 in the manufacture of cakes of the drop-cake machine type. The first had an ash content of .50 per cent and a protein content of 9.12 per cent, while the latter had an ash and protein content of .49 and 8.94 per cent, respectively. These data again indicate that so far as is shown by average values, there was little difference between the grade (as determined by ash content) and the strength (as determined by protein content) of flours used for the manufacture of the types of biscuit considered in Table III. Thus the average protein content of the flours used in these several types of goods varied between 8.92 and 9.59 per cent, while the average ash content varied between .49 and .51 per cent. These differences appear too small to determine the type of product for which the flour is to be used. In individual instances certain plants may have used flours of different strength and grade for the several purposes, but such differences disappear in the average.

Viscosity studies.—Not only is the concentration of gluten in flour of significance in relation to baking strength, but the "quality" of the gluten is of equal importance. While several methods have been suggested for determining the properties of gluten which are correlated with its quality, the procedure developed by Sharp and Gortner (1923) is probably the most rational and exact. They found that the increase in viscosity of acidulated water suspensions of leached flour effected by each unit increase of flour in the suspension, was correlated with gluten quality. Thus, plotting the logarithm of viscosity of the acidulated suspension as ordinates against the logarithm of the concentration of flour in the suspension as abscissas, gives a straight line. The tangent of the angle which this curve forms with the horizontal is a convenient expression of the characteristics of the curve, and is positively correlated with gluten quality. It is obvious that not less than 4 or 5 determinations must be made, using a different ratio of flour to water in each instance, in order to plot such a curve. The procedure is described in detail by Gortner (1924) and need not be repeated here.

The quality constants (b) derived by each series of viscosity measurements of more than half the flour samples are given in Table III. These constants are remarkably high, averaging considerably greater than the quality constants similarly derived, and reported by Sharp and Gortner (1923) (Table XIV, p. 67). The maximum constant reported in this table in the paper by Sharp and Gortner is 3.6, while constants in excess of 3.6 are not uncommon in the case of the soft wheat flours which were examined.

These data further emphasize the contention of Gortner (1924) that the determination of gluten quality can not be confined to a single viscosity measurement. This is shown by the data recorded in Figure 3, resulting from a study of five flours, Nos. 32, 101, 103, 118, and 119. The actual magnitude of the readings in these cases is not correlated with the tangent of the angle which the curve forms with the horizontal or the quality constant (b), and no less than four concentrations of flour must be employed accurately to determine the latter.

In the discussion of the viscosity measurement which has been indulged in by several chemists, mention has frequently been made of the desirability of applying the test to an unleached flour suspension in which all the electrolytes of the flour extract are present. To ascertain the relation between the viscosity of leached and unleached flour suspensions after acidulation, a series of comparisons was made, using 14 flour samples. In the case of the unleached suspensions, the desired quantity of flour was weighed into a flask and 100 cc. of water added with constant agitation to produce a smooth, homogeneous suspension. This was allowed to stand exactly one hour and then swelled with lactic

acid. It was necessary to use concentrated, syrupy lactic acid (75%) to effect the maximum swelling without too great dilution. A comparison of the determination made with the electrolytes present (unleached) and the electrolytes removed (leached) is afforded by the data in Table IV. A study of these data shows no correlation between the two sets of determinations in so far as the quality constant is concerned. The electrolytes, when present, exert a varying effect on the quality constant, and hence must be removed when this constant is to be determined. There is, moreover, no evidence that the difference in the quality constant in the presence and absence of the electrolytes is of significance in affording a measure of the effect of these electrolytes on the properties of the gluten, or of an ordinary dough in which they are present.

TABLE IV

Comparison of Viscosity Data on the Same Flours with Electrolytes Present and Electrolytes Removed

No.	Treatment		Ash %	Protein %	Constant b	Viscosity at different flour concentrations					
						15	18	21	24	27	30
1	Electrolytes	present	.57	7.92	3.21	9	15	24	38	58	81
	"	removed			3.15	34	62	99	146		
2	"	present	.44	9.80	3.71			35	61	94	131
	"	removed			3.81	25	56	97	151		
3	"	present	.42	8.15	3.70			24	41	62	90
	"	removed			3.02	24	59	82	112		
4	"	present	.52	9.41	3.56			30	47	73	106
	"	removed			3.35	34	68	111	165		
5	"	present	.40	8.38	4.09			31	58	75	144
	"	removed			3.49	24	47	74	128		
6	"	present	.40	8.38	3.85			27	53	74	110
	"	removed			3.15	36	76	107	165		
7	"	present	.51	8.44	3.76			21	34	55	79
	"	removed			3.92	24	56	94	155		
8	"	present	.51	8.61	3.24		11	16	23	38	57
	"	removed			4.00	14	27	57	88		
9	"	present	.48	8.95	3.10			34	59	80	104
	"	removed			3.73	29	67	90	185		
10	"	present	.43	9.46	3.15			36	65	92	110
	"	removed			3.22	48	80	137	207		
11	"	present	.41	8.21	2.86			34	51	67	98
	"	removed			3.22	44	80	132	200		
12	"	present	1.71	13.11	3.69			16	28	38	62
	"	removed			2.57	34	55	80	115		
59	"	present	.49	8.72	2.79	10	18	24	39		
	"	removed			3.34	26	47	78	114		
80	"	present	.53	9.29	3.64	8	16	32	42		
	"	removed			3.06	30	59	89	129		

Changes in hydrogen-ion concentration which occur during the fermentation process and subsequent baking of the dough into crackers.—Bailey and Sherwood (1923) followed the increase in hydrogen-ion concentration in bread dough during the fermentation period and note that in well regulated bakeries the rate of such changes

is constant from day to day. They also note that the bread produced in such bakeries had an almost constant pH value over the period of time during which observations were made. This work suggested that similar methods of study might be applied to cracker doughs in order to follow the rate of fermentation. Such work would perhaps indicate the applicability of hydrogen-ion determinations as a means of control, or suggest methods of control which would be applicable to such a process.

Despite extensive investigations of alcoholic fermentation there is still a lack of agreement as to the exact succession of chemical changes occurring in this complex phenomenon. Neuberg and Hildescheimer (1911), and Kostytschev (1912) found that pyruvic acid is fermented by yeast more readily than sucrose. This observation led to the suggestion that several reactions were involved in the formation of the principal end products of fermentation—alcohol and carbon dioxide.

Thus Kostytschev (1912) suggested that fermentation might involve the following reactions:

(1) $C_6H_{12}O_6 \rightarrow 2CH_3COCOOH + 4H$

(2) $2CH_3COCOOH \rightarrow 2CH_3CHO + 2CO_2$

(3) $2CH_3CHO + 4H \rightarrow 2C_2H_5OH$

Reaction 1 involves the splitting of the hexose molecule into pyruvic acid and hydrogen. Then a carboxylase, which Neuberg and Hildescheimer (1911) have shown to be present in yeast, acts on the pyruvic acid to form acetaldehyde and carbon dioxide, according to equation 2. Hahn (1903) had previously observed that yeast reduced methylene blue and had ascribed this reducing action to a reductase present in the yeast. Kostytschev, therefore, postulated the reduction of the acetaldehyde by this enzyme to form alcohol, in accordance with Equation 3.

Neuberg and Kerb (1913) proposed a more elaborate mechanism than this, the oxidations and reductions being based on the Cannizzaro reaction. These workers believe that the hexose molecule is split into two molecules of methylglyoxal with the loss of water, according to the following reaction:

$C_3H_{12}O_6 \rightarrow 2CH_3COCHO + 2H_2O$

A portion of the aldehyde thus formed is converted into glycerol and pyruvic acid by a Cannizzaro reaction.

$2CH_3COCHO \rightarrow CH_2OHCHOHCH_2OH + CH_3COCOOH$

The pyruvic acid is then decomposed by the carboxylase already mentioned, according to the following reaction:

$CH_3COCOOH \rightarrow CH_3CHO + CO_2$

A molecule of the acetaldehyde and a molecule of methylglyoxal undergo another Canizzaro transformation, to yield alcohol and pyruvic acid, which acid is in turn decomposed by the carboxylase.

$$CH_3COCHO + CH_3CHO \rightarrow CH_3CH_2OH + CH_3COCOOH$$

A small quantity of glycerine may thus be formed, as is actually found to be the case in fermentation. Neuberg (1922), in a review of his work, states that since this theory was proposed some of the reactions have been proved to occur during fermentation while others, altho not established, have not been shown to be impossible.

There is some doubt, however, as to the possibility of an accumulation of pyruvic acid in a fermenting cracker dough. Neuberg does not believe that so reactive a substance can exist in the presence of active yeast cells. Fernbach and Schoen (1922-23) believe, however, that there is always a determinable quantity of pyruvic acid present in fermenting media. These investigators have isolated the calcium salt of this acid, as well as that of lactic acid, from fermenting media. Neuberg claims, on the other hand, that the calcium salt of pyruvic acid was fixed by the reaction of the acid with added chalk, and pyruvic acid derivatives accumulate only when some fixing reagent is present. Thus it is obvious that pyruvic acid is perhaps not the acid to which the greatest increase in the acidity of a fermenting mass is due, altho the most plausible theories of the mechanism of alcoholic fermentation consider this acid an intermediate product.

Lactic acid was at one time held by many biological chemists to be an intermediate product in the fermentation of sugar. The fact that lactic acid is fermented to alcohol and carbon dioxide only with considerable difficulty, militates against the acceptance of this theory. Thus Furth and Sieben (1922) state that yeast suspensions through which a current of oxygen is passed are able to decompose only about one-half the quantity of added lactic acid. No alcohol is formed. These workers suggest that the rest of the lactic acid molecule is utilized by the yeast in building tissue. Buchner and Meisenheimer (1909-10) even go so far as to state that lactic acid is neither formed nor fermented by pure yeast cultures. Most investigators believe, however, that traces of lactic acid are formed as a side reaction to alcoholic fermentation. As the acid is not decomposed during fermentation, it accumulates and tends to increase the hydrogen-ion concentration of the medium.

Another acid which is generally found in fermenting material is acetic acid. Neuberg (1922) states that if the optimum acidity for fermentation be maintained, carbon dioxide and alcohol only will be formed. If the medium be on the alkaline side of the optimum reaction

for fermentation, acetic acid may be formed, according to the following equation:

$$2C_6H_{12}O_6 + H_2O = 2CO_2 + C_2H_5OH + CH_3COOH + 2C_3H_8O_3$$

This equation conforms to Neuberg's third form of fermentation.

Fermentation with yeast also results in the production of several other acids. Chief among these are succinic and glutaric acid. These acids are formed by the deamidization of the corresponding mono-amino acids, aspartic and glutamic acids.

Not only does the metabolism of the yeast result in an increase in the hydrogen-ion concentration of such a medium as a dough, but also the micro-organisms present in the flour and those added with the yeast may also effect the same result. Thus Effront (1917) states that flour may contain as many as 30,000 bacteria per gram. It is probable that micro-organisms other than yeast play a part in the production of acids in a cracker dough during fermentation with baker's yeast. It is also probable that these micro-organisms produce lactic acid, acetic acid, and perhaps small quantities of butyric acid, that is, acids of the same type as are produced by the yeast itself. The total quantities of these acids and their effects on the physical properties of the dough will be discussed later in this paper.

A detailed study of certain chemical and physical changes occurring in cracker sponges and doughs was made in a commercial cracker bakery. There are two distinct stages in the process as conducted in this and most other cracker bakeries, involving the "sponge" and the "dough" stages. In preparing the sponge, the following materials were placed in the mixing machine:

	Pounds
Flour	868
Lard	40
Yeast	1
Water	300
Total	1209

These were mixed for four minutes and the sponge thus formed was allowed to ferment for 18 hours. The temperature of the freshly mixed sponge was usually about 22°C. (72°F.). The temperature increased, slowly at first, but more rapidly as fermentation proceeded, until at the end of the 18-hour period the temperature of the dough was about 30°C. (86°F.). The fermentation was carried on in a room in which the temperature was maintained at 27°C. (81°F.). Hence the higher temperature of the room partially accounts for the rise in temperature of the dough. Yeast fermentation, however, is an exothermic process and it is to heat derived from this process that most of the rise in temperature is due. At the end of the 18-hour fermer

tation period the following ingredients were added to the sponge to form what is known as the "dough":

	Pounds
Flour	112
Lard	40
NaCl	12
$NaHCO_3$	6-10

The materials which make up the dough were mixed for 10 minutes and fermentation was allowed to proceed for from 3 to 6 hours longer, at the end of which time the dough was rolled into thin sheets and baked into crackers.

During this long fermentation period many changes occurred in the dough. Owing to metabolic processes of the yeast itself and of other organisms present in the yeast and flour, the dough became distinctly acid. Coincident with this increasing acidity were other changes. Our attention for the present will be confined to the significance of this increased acidity and the problems connected with it.

In Table V are presented detailed data which show the reaction of the sponge, dough, and crackers throughout the process of cracker manufacture. The dough suspensions used for the determination of the hydrogen-ion concentration were prepared in the following manner: Fifteen grams of dough was broken into small bits and dropped into a 100 cc. test tube. Fifty cc. of distilled water was added and the stoppered tube shaken vigorously for 4 to 5 minutes, or until most of the dough was disintegrated and the flour particles suspended in the water. A portion of this suspension was poured into a Bailey cell and the hydrogen-ion concentration determined by the electrometric method, as in the work on crackers. In the case of doughs Nos. 1 and 5, the hydrogen-ion concentration, in terms of Ch $\times$ 10^{-5}, as well as in terms of pH, is given in the table. These data indicate that during the first 4 to 6 hours there was only a slight increase in hydrogen-ion concentration; the most important change in progress was the hydration of the gluten and other flour constituents. After 6 to 8 hours the production of acid increased at an accelerated rate. Thus in dough No. 1, the hydrogen-ion concentration only approximately doubled during the first 8 hours, while during the next 11 hours it increased to more than 20 times its value at the eighth hour. The same phenomenal increase in acidity was noted in No. 5. During the first 6 hours the hydrogen-ion concentration increased from .153 $\times$ 10^{-5} to .296 $\times$ 10^{-5}, while in the next 7 hours it became 6.42 $\times$ 10^{-5}, again an increase of 20 times its value at 6 hours.

There may be several reasons for the greater activity at this time. The short and consequently imperfect mixing of the materials does not allow them to become thoroly or uniformly wetted, and in this poorly

wetted condition the dough does not furnish the optimum medium for the metabolism of the organisms. While the great increase in rate of acid production can not be ascribed to any single cause, it appears that the mere wetting or hydration of the material is an important factor in rendering the dough a more suitable medium for the growth of organisms. Then, with a more suitable medium there results an increased metabolic activity as well as increased numbers of organisms metabolizing owing to more rapid reproduction. The fact that slacker, less viscous sponges begin to develop acid earlier in the fermentation process substantiates this contention. If the increase in rate of acid formation were due to increased number of organisms alone, then this increase would be geometric. This, however, is not in accordance with the facts. Another factor which may have a bearing on the rate of acid production is hydrogen-ion concentration itself. As fermentation progresses the hydrogen-ion concentration approaches more nearly the optimum for the activity of certain organisms. In the later stages of fermentation the continuing increase in concentration of hydrogen-ions operates to retard acid production. In one experiment a portion of dough was allowed to ferment for 48 hours. During the last 20 hours there was practically no change in hydrogen-ion concentration or in titratable acidity. In another instance a dough which at the end of 24.5 hours had a hydrogen-ion concentration of $.102 \times 10^{-3}$ was allowed to ferment for 144 hours longer. At the end of this time the hydrogen-ion concentration had only increased to $.144 \times 10^{-3}$, which is an almost insignificant change when compared with the greater changes which take place in the early stages of fermentation. That this apparently slight change is not due to the depression of ionization by the weak acids formed, is indicated by titration. The quantity of .02 normal sodium hydroxide required to neutralize the acids in 3.2 grams of the above dough increased from 12.6 cc. to 13.4 cc. during this 144-hour period. This slight increase of .8 cc. indicates that when the fermenting dough has developed a certain quantity of acid, this high acidity operates to retard further acid production. That it is the acid and not some other metabolic product which retards the increase in acidity in the acid doughs, is indicated by the fact that when such doughs are partially neutralized with sodium bicarbonate, the same rapid rate of acid production takes place again but the rate falls off as the dough approaches the original acid condition. These observations are plotted in terms of pH in Figure 4, pH being plotted rather than hydrogen-ion concentration because of the difficulty of plotting the great changes in hydrogen-ion concentration which occur. Thus, in this work the pH range lies between pH 4.0 and pH 9.0, approximately. Hence, in such a case

the acid dough has 100,000 times the hydrogen-ion concentration of the baked cracker.

A study of Table V and Figure 4 indicates that there is considerable variation both in the rate of development of acid and in the final quantity of acid present in the dough. Thus in the case of dough No. 9 the final hydrogen-ion concentration of the sponge before the addition of sodium bicarbonate was 1.46×10^{-5}, while dough No. 5 at this same stage in point of time fermented (19 hours of fermentation) had a hydrogen-ion concentration of 14.55×10^{-5} and increased to 18.4×10^{-5} before the sodium bicarbonate was added. Hence the second dough had a hydrogen-ion concentration more than 10 times as great as the first one. In the neutralization of sponges differing to such an extent as these, errors in the quantity of sodium bicarbonate used often occur. The quantity of bicarbonate to be added is left to the judgment of experienced men who base their estimate on the temperature and feeling of the dough. That errors in judgment do occur is also seen in the data in Table V. Nine pounds of sodium bicarbonate added to dough No. 5 brought the pH to 6.98, while 9½ pounds of the bicarbonate added to dough No. 6 brought the pH to 7.51. The data from the study of doughs Nos. 5 and 6 are plotted in Figure 4. Dough No. 5 became acid very rapidly in the interval of time between the addition of the soda and the baking of the crackers. Dough No. 6 changed only slightly during the same period of time. Crackers baked from the more acid dough had the same pH as the dough from which they were baked, while the crackers baked from the alkaline dough were much more alkaline than the dough. This increase in alkalinity was due to processes which take place during the baking. The bicarbonate was converted to the more alkaline carbonate and the acid-reacting carbon dioxide was expelled from the dough solution by the heat of the baking process.

According to the practical man, the crackers produced from both of these doughs were undesirable, the one was too acid, the other too alkaline. Between these two limits must lie the ideal product which the industry desires.

Of all the doughs listed in Table V, only Nos. 4 and 7 produced crackers which approached the ideal type desired. These doughs, after the addition of bicarbonate, were slightly on the alkaline side of neutrality. A pH of 7.10 at this stage of the process appeared to be the optimum for the production of the best crackers. Before discussing the development of a method for determining the quantity of bicarbonate to be added in order that the dough be brought to this pH, the changes which take place in the cracker during the baking process will be discussed in more detail.

TABLE V

CHANGE IN HYDROGEN-ION CONCENTRATION, AS SUCH AND IN TERMS OF pH, AS IT OCCURS IN CRACKER MANUFACTURE, DURING THE FERMENTATION OF THE SPONGE, THE NEUTRALIZATION OF THAT SPONGE WITH SODIUM BICARBONATE, AND THE BAKING OF CRACKERS FROM THE DOUGH THUS FORMED

Dough No. 1			Dough No. 2		Dough No. 3		Dough No. 4		Dough No. 6		Dough No. 7	
Time Hrs.	pH	CH x 10^{-5}	Time Hrs.	pH	Time Hrs.	pH	Time Hrs.	pH	Time Hrs.	pH	Time Hrs.	pH
0	5.85	.141	0	5.85	0	5.85	0	5.83	0	5.80	0	5.83
2	5.80	.165	2	5.75	2	5.76	2	5.71	2	5.76	2	5.73
4	5.63	.235	4	5.68	4	5.68	4	5.61	4	5.66	4	5.60
6	5.58	.264	6	5.68	6	5.60	6	5.60	6	5.61	6	5.54
8	5.49	.320	6:10	5.61	8	5.51	8	5.49	6:05	5.43	8	5.44
10	5.20	.621	8	5.54	10	5.27	10	5.20	8	5.44	10	5.22
12	4.92	1.200	10	5.34	12	4.83	12	4.87	10	5.29	12	4.76
14	4.51	3.060	12	5.09	14	4.56	14	4.51	12	5.00	14	4.55
19	4.16	6.940	12:30	5.05	16	4.36	19:10	4.04	17	4.65	16	4.29
21:10	4.07	8.430	14	4.80	19:20	4.16	21	3.94	19:10	4.19	19	4.07
23:30	4.02	9.470	19	4.26	19:30	7.15	21:10	7.22	21:30	4.09	20	4.04
24:40	3.99	10.200	21:20	4.12	19:50	7.08		7.18	22:40	4.04		7.25
25:10	7.41	.00394	23	4.05	21:50	7.20	23:20	7.13	22:50	4.04	22	7.22
	7.47	.00337	23:40	7.25		7.17		7.08	22:50	7.51	24	7.01
28:10	7.37	.00426		7.24	23:40	7.07	25:30	6.88	23	7.47	26:20	6.71
	7.35	.00443	25:40	7.20		7.01		6.90	25:20	7.37	Crackers	7.55
29:05	7.28	.00517		7.17	Crackers	8.38	Crackers	7.74	25:40	7.39		
	7.25	.00559	Crackers	8.54					26:45	7.33		
Crackers	8.96	.000110							Crackers	9.18		
10 lbs. $NaHCO_3$ used			8.5 lbs. $NaHCO_3$ used		8.75 lbs. $NaHCO_3$ used		7.5 lbs. $NaHCO_3$ used		9.5 lbs. $NaHCO_3$ used		8.5 lbs. $NaHCO_3$ used	

TABLE V—*Continued*

Dough No. 5			Dough No. 8		Dough No. 9		Dough No. 10		Dough No. 11		Dough No. 12	
Time Hrs.	pH	CH x 10^{-5}	Time Hrs	pH	Time Hrs	pH	Time Hrs.	pH	Time Hrs.	pH	Time Hrs.	pH
0	5.82	.153	0	5.91	0	6.00	0	5.83	0	5.78	0	5.83
2	5.78	.165	2	5.81	2	5.92	2	5.73	2	5.68	2	5.57
4	5.63	.209	4	5.73	4	5.83	4	5.63	4	5.64	4	5.60
6	5.53	.296	6	5.65	6	5.75	6	5.56	6	5.58	6	5.58
8	5.02	.953	8	5.54	8	5.65	8	5.49	8	5.48	8	5.46
8:10	4.99	1.030	10	5.48	10	5.56	10	5.44	10	5.24	10	5.27
8:40	4.87	1.350	12	5.41	12	5.48	12	5.32	12	4.95	12	5.19
10	4.46	3.440	14	5.25	14	5.32	14	5.27	14	4.63	14	5.07
12	4.31	4.890	16	5.05	18:30	4.83	19	4.83	19	4.16	19	4.45
13	4.19	6.420	19:30	4.75	18:40	7.28	21	4.68	21	4.09	21	4.43
14	4.11	7.800		7.39	20	7.24	23	4.34	23	4.01	23	4.29
19	3.84	14.550	21:30	7.35	22	7.20		7.30		7.18	24:20	4.14
21:20	3.77	17.300	23:30	7.32	Crackers	9.33	25:10	7.27	25:20	7.15	24:30	7.42
23:10	3.73	18.400	25	7.17			27	7.27	27:20	7.12	26:30	7.35
23:20	6.98	.0104	Crackers	9.31			Crackers	9.21	Crackers	8.70	28:30	7.27
	6.96	.0108									Crackers	9.21
27	6.02	.0958										
Crackers	5.95	.1120										
9 lbs. $NaHCO_3$ used			8 lbs. $NaHCO_3$ used		7.5 lbs. $NaHCO_3$ used		8 lbs. $NaHCO_3$ used		9 lbs. $NaHCO_3$ used		8.5 lbs. $NaHCO_3$ used	

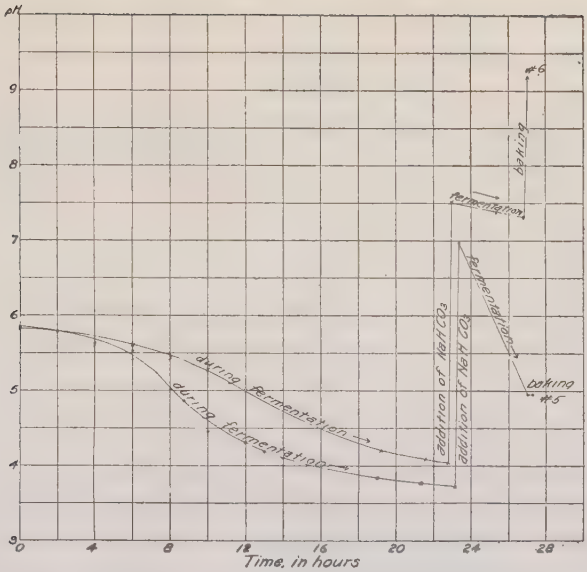

Fig. 4. Changes in Hydrogen-ion Concentration (in terms of pH) which Occur During the Fermentation of Sponge, Fermentation of the Dough Subsequent to Neutralization of Sponge with $NaHCO_3$, and During the Baking Process

Changes in hydrogen-ion concentration which take place in the cracker during the baking process.—It was noted in the previous section that the dough which was only slightly alkaline produced crackers which were considerably more alkaline. This change was no doubt due to the conversion of bicarbonate to carbonate and the loss of the acid-reacting carbon dioxide from the dough during the baking process. Both of these reactions so operate as to increase the alkalinity of the baked cracker. The reaction which plays the more prominent part is the conversion of bicarbonate to carbonate, according to the following reaction:

$$2NaHCO_3 \xrightarrow{\text{heat}} Na_2CO_3 + CO_2 + H_2O$$

That this is actually the case is shown by the fact that the baked cracker often has a hydroxyl-ion concentration greater than could exist even in a saturated solution of sodium bicarbonate. Considerations based on the conversion of bicarbonate to carbonate will therefore be presented in this section. Table VI gives the pH of a whole series of doughs of increasing alkalinity and the corresponding pH of the crackers baked from such doughs. The cracker extracts, the hydrogen-ion concentrations of which were determined, were prepared in the manner already described. It should be noted that the crackers used for the determination were not taken from the outer, more-

browned rows of the peel but from the inner rows. These data show that the greater the alkalinity of the dough, the greater the increase in alkalinity when such doughs are baked into crackers.

TABLE VI

HYDROGEN-ION CONCENTRATION AS pH OF THE DOUGH IMMEDIATELY BEFORE BAKING, pH OF THE BAKED CRACKER, AND INCREASE IN pH DUE TO BAKING

pH of dough	pH of cracker	Increase in pH due to baking
7.41	9.43	2.02
7.28	9.35	2.07
7.27	9.21	1.94
7.27	9.21	1.94
7.25	8.96	1.71
7.20	8.58	1.38
7.17	8.54	1.37
7.01	8.38	1.37
6.97	8.12	1.13
6.90	7.74	.84
6.71	7.55	.84
5.73	6.03	.30
6.02	5.95	—.07

The data in Table VI indicate that the extent of change of hydrogen-ion concentration which takes place during the baking process is not always uniform. Other unpublished data show the same exceptions with regard to extent of change. In one case a dough of pH 7.17 gave a cracker of pH 9.31, while in the last case cited in the table the baked cracker was more acid than the dough from which it was baked. These observations suggested that some reaction other than the bicarbonate to carbonate conversion was involved in effecting changes in hydrogen-ion concentration during the baking process. It had already been noted that the cracker extracts having the darkest color were likewise the most alkaline. It was further observed that when a rather alkaline dough was baked, the outside rows of the pan or peel were browner than the inner rows. This led to the determination of the hydrogen-ion concentration of the several rows of crackers, with the results shown in Table VII. The rows were numbered from edge to edge of the sheet of dough, there being 14 rows in all. As might be supposed, the hydrogen-ion concentration of the 12 inner rows was practically uniform. The two outer rows, Nos. 1 and 14, were, however, appreciably less alkaline than the inner rows. These two outer rows were likewise more heavily browned.

TABLE VII

HYDROGEN-ION CONCENTRATION AS pH OF CRACKERS TAKEN FROM EACH ROW ACROSS THE PEEL OF CRACKERS

Row	pH
1	8.27
2	8.99
3	8.99
4	9.08
7	9.01
9	9.03
12	9.11
13	9.11
14	8.74

To accentuate further the effect of location, several lots of crackers were baked a second time. The hydrogen-ion concentration of the inner and outer rows of four lots of crackers of varying alkalinity and of the same crackers baked a second time, are given in Table VIII. From these data it is evident that in the case of crackers baked once in the usual manner, the difference in the hydrogen-ion concentration of the inner and outer rows of the same batch increases with the alkalinity of the inner row. Likewise the proportional decrease in alkalinity as the result of the second baking is greater in case of the alkaline crackers. In the case of the two lots of crackers which were acid after a single baking, there was no change in hydrogen-ion concentration as a result of rebaking.

The reason for this peculiar change in alkalinity of the biscuit is probably to be found in a series of reactions. That the temperature of the baking process is high enough to effect a conversion of bicarbonate to carbonate is evident from the fact that the cracker oven is maintained at a temperature in excess of 270°C., which is the temperature at which sodium bicarbonate loses CO_2, according to Olsen (1913, p. 191). Crackers become so dry in the baking process as to reach a temperature on the crusted surface closely approaching that of the oven. This loss of CO_2 from bicarbonate would account for an increase in alkalinity of the dough during baking. The lower alkalinity of those crackers subjected to the more extensive treatment with heat, either because of their location along the outer edge of the sheet or because of a second baking, presumably results from the effect of the alkali on the sugars present in the dough. According to Nef (1913), sugars heated in an alkaline medium are converted into a variety of substances, including a group of organic acids. These acids then form salts with the alkali, thus neutralizing the latter, and reducing the alkalinity of the mixture. Such reactions may conceivably occur in cracker dough under the conditions just detailed. While the crackers which were acid in reaction will brown when heated vigorously, they change little in degree of acidity. Probably the acids formed by heating the sugars in such cases are not ionized sufficiently to increase the hydrogen-ion concentration.

TABLE VIII

EFFECT OF BAKING PROCESS ON HYDROGEN-ION CONCENTRATION OF SEVERAL LOTS OF CRACKERS

Treatment	Hydrogen-ion concentration			
	Dough No. 13 pH	Dough No. 14 pH	Dough No. 12 pH	Dough No. 15 pH
Single baking				
Inner rows	8.94	7.86	6.24	....
Outside rows	7.88	7.59	6.17	6.03
Double baking				
Inner rows	7.30	7.10	6.20	....
Outside rows	6.68	6.68	6.20	5.97

Chemical control of the neutralization of the acids produced during fermentation.—In a previous section it was noted that the crackers which the practical man considered as ideal were produced from doughs which had been brought to a pH of 6.95 to 7.10 by the addition of sodium bicarbonate. When such doughs were baked, the crackers were slightly more alkaline than the dough. There are several reasons for the desirability of a slight excess of bicarbonate in the dough over that required to neutralize the acids of fermentation. A slight excess of soda results in the development of a certain degree of brownness in the baked cracker. Moreover, light, brittle crackers are produced when the dough contains an excess of soda. The lightness is due, obviously, to the carbon dioxide set free during the baking process which serves to inflate the cracker.

Acid crackers, on the other hand, are generally regarded as less desirable for several reasons. Such crackers are usually less brown and lack the "bloom" of the slightly alkaline crackers. Acid crackers are also inclined to be less crisp, owing probably to the fact that the reaction of such crackers during the baking departs less from the isoelectric point of the gluten proteins than is the case with crackers baked from a slightly alkaline dough. While Sharp and Gortner (1923) have presented data (see p. 61 of their bulletins) which show an "isoelectric range" for wheat glutenin, it is evident that the pH of crackers baked from a slightly alkaline dough approaches the upper limit (in terms of pH) of this range. Bailey and Le Vesconte (1924) have also shown that the extensibility of dough diminishes rapidly on progressively rendering the dough alkaline. At the isoelectric point the gluten proteins are presumed to be hydrated less, and in a tougher, more tenacious condition than is the case when the alkaline limit of the isoelectric range is approached or exceeded. It should accordingly be expected that an acid dough, the hydrogen-ion concentration of which is near the mean of the isoelectric range and remains stationary during the baking, should yield a tougher cracker than a dough the alkalinity of which increases and approaches or exceeds the limits of the isoelectric range during the process of baking.

Crackers with too great an excess of bicarbonate possess undesirable properties, however, and in such instances as dough No. 6, graphed in Figure 4, care must be taken in regulating the heat or the crackers will become exceedingly brown and assume the appearance of having been burned. Because of the excess carbonate, the crackers produced from an alkaline dough may have a smaller specific volume than is desirable. In such instances difficulty will be encountered in packing the desired weight of crackers in a standard size container. In the larger cracker boxes this deficiency in weight due to the over-expanded crackers

may be as much as two or three pounds. Likewise, doughs without the proper excess of bicarbonate are too heavy and when the proper weight is packed in the standard container, the container is not full. The regulation of alkalinity is accordingly important to insure a uniform net weight of the contents of cracker packages.

In controlling the alkalinity of crackers, one method that may be followed is to titrate a suspension of the dough to a pH of 7.10, assuming this to be the degree of alkalinity which a dough should have for the production of the ideal cracker. From the quantity of standard alkali used in the titration, the proper quantity of bicarbonate to neutralize the acids due to fermentation can be calculated. As a portion of the flour is yet to be added, the quantity of alkali required to bring this flour to the same pH must be determined.

In conducting a series of electrometric titrations of the dough, 32 grams of the dough was shaken until a smooth suspension was obtained. These suspensions were made up to 250 cc. in a volumetric flask. Aliquots of one suspension were taken and 0.02 N sodium bicarbonate was added so that each aliquot would contain the standard bicarbonate solution in the ratio of 10, 20, 30, 40, 50, 60, or 70 cc. per 100 cc. of the suspension. In a similar manner aliquots of another suspension, from another dough, were prepared so that each aliquot would contain 10, 20, 30, 40, or 50 cc. of 0.02 N sodium hydroxide per 100 cc. solution. Thus each 100 cc of dough suspension was equivalent to 12.8 grams of dough. In preparing the dough suspensions for the electrometric titrations, an effort was made to use approximately the same concentration of dough in suspension as was used for determination of hydrogen-ion concentration of the dough. This accounts for the odd value of 12.8 grams of dough per 100 cc. of dough suspension. The pH of the suspensions prepared in this manner was determined electrometrically by use of the apparatus already described. An effort was made to use the cadmium sulfate–potassium iodide half cell described by Sharp and MacDougall (1922). Such a half cell, which had a difference of potential equivalent to a pH of 7.10 against a normal calomel half cell, was prepared. It required too long, however, for the Bovie (1922) electrode to become saturated with hydrogen, and for this reason such a method was discarded in favor of the Bailey electrode already mentioned. In Table IX and Figure 5 are recorded the resulting data.

TABLE IX

ELECTROMETRIC TITRATION OF ACID DOUGH SUSPENSIONS WITH 0.02 N SODIUM BICARBONATE AND SODIUM HYDROXIDE

.02 N NaOH per 100 cc. of dough suspension	pH	.02 N $NaHCO_3$ per 100 cc. of dough suspension	pH
cc.		cc.	
0	3.92	0	3.76
10	4.45	10	4.68
20	5.31	20	6.04
30	6.52	30	6.63
40	7.18	40	6.86
50	8.18	50	7.01
		60	7.12
		70	7.20

If the quantity of sodium bicarbonate sufficient to bring the dough, portions of which had been neutralized with sodium bicarbonate, to pH 7.10 be calculated from the data in Table IX, discrepancies appear between the quantity of bicarbonate to be added to bring the doughs to this pH and that required to bring the solutions to the same pH. Calculating on the basis of the quantity of 0.02 N sodium bicarbonate used, 9½ pounds of bicarbonate should be required to neutralize the acids of fermentation. Actually 8¼ pounds were added in the preparation of the dough; and the man in charge considered this quantity to have been about 3 pounds too little, as the crackers produced from this dough were exceptionally acid. The pH of the crackers was 5.19. Calculating on the basis of the quantity of 0.02 N sodium hydroxide required to bring the other dough suspension to pH 7.10, about 6.5 pounds of sodium bicarbonate should have been required to bring the dough to the same pH. Actually, however, 10 pounds were used but the crackers were considered too alkaline. These crackers had a pH of 8.52.

It might be assumed that titration with standard bicarbonate solution would probably give results more in accordance with what is desired. It was found, however, that there was too little change in pH over the critical portion of the titration curve to give clear cut results. In Figure 5 it is shown that 20 cc. of standard bicarbonate solution is required to effect a change of .20 pH units. In terms of bicarbonate per sponge, the preparation of which has been described, this difference is equal to about three pounds. Hence the use of standard bicarbonate solution was not considered advisable because a sharp enough end point could not be obtained.

The flattening out of the titration curve in this region was probably due to the liberation of carbon dioxide by the reaction between the acids of fermentation and the bicarbonate, and a consequent higher acidity due to the solution of the carbon dioxide in the mixture. In

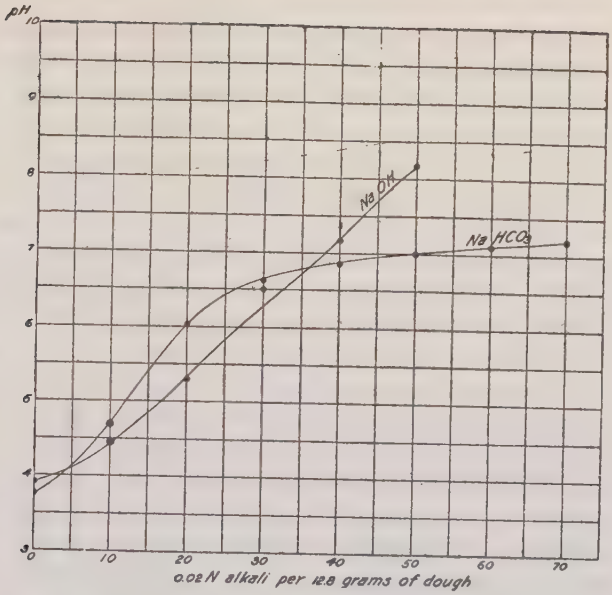

Fig 5. Electrometric Titration Curves of Acid Sponge Suspensions with 0.02 N Sodium Hydroxide and Sodium Bicarbonate

order to ascertain what was happening in the neutralization of the dough, a series of observations was made from which lactic acid titration curves with both sodium hydroxide and sodium bicarbonate were prepared. In developing the lactic acid–sodium bicarbonate titration curves, two solutions were used from which (a) the carbon dioxide had been swept out by a stream of hydrogen, and (b) the carbon dioxide was not thus removed. These data are presented in Table X and the titration curves are shown in Figure 6. To 25 cc. of 0.02 N lactic acid were added the quantities of alkaline solution indicated in the table and the hydrogen-ion concentration was determined. In preparing the flour suspension, an effort was made to obtain a suspension of the same flour-concentration and pH value as was obtained from the fermenting dough. A liter of flour-suspension was prepared in such a manner as to contain the equivalent of 12.8 grams of cracker dough per 100 cc. of suspension. This suspension was acidified with 20 cc. of normal lactic acid and allowed to stand for one hour. At the end of this time, 25 cc. aliquots were taken for the electrometric titration.

A study of Figure 6 indicates what occurs in the neutralization of the acids of the dough. Carbon dioxide is liberated by the reaction between acid and bicarbonate. The carbon dioxide so formed tends to increase the hydrogen-ion concentration. Hence a determination

of the hydrogen-ion concentration of the neutralized dough does not indicate whether there is excess carbonate present or not, judging from the pH value alone. It is probable, however, that the dough retains less carbon dioxide than the solutions which were used, but even this assumption does not make for results which are sufficently accurate. Thus, when it was stated that the optimum pH of the dough for the production of good crackers was 7.10, this means the apparent pH in the presence of the carbon dioxide of the dough solution. When the carbon dioxide has been removed by aspiration, actual excess of sodium bicarbonate over that required to neutralize the acids of fermentation will be indicated in a more exact manner.

It is interesting to note that the pH of the solutions neutralized with sodium bicarbonate and sodium hydroxide is the same until these substances themselves begin to affect the hydrogen-ion concentration.

The acidified flour–sodium hydroxide titration curve appears to run parallel to the sodium hydroxide–lactic acid curve on the acid side of neutrality. This may indicate that we are titrating lactic acid only or substances which ionize to the same degree as lactic acid. Sufficient lactic acid had been added to the flour suspension to make a 0.02 normal solution. It is probable that a part of this acid was bound by the proteins of the flour or absorbed by other substances present in the flour suspension, and when this flour was titrated only the unbound portion is free, so that actually we are titrating lactic acid only. On the alkaline side of neutrality the buffer materials of the flour operate to reduce the pH value of the flour suspensions which had been acidified. It is possible that if a suspension of flour had been rendered alkaline and then titrated with lactic acid, the curve would run parallel to the lactic acid–sodium hydroxide curve. Such data as these may be of significance in determining the type of acids produced during fermentation.

An effort was next made to determine the hydrogen-ion concentration of the dough solutions after the removal of the carbon dioxide by aspiration with hydrogen. The data given in Table XI indicate the results which were obtained. The dough suspensions used in this work were prepared in the same manner as those described in a previous section, using 32 grams of dough shaken with distilled water until a smooth suspension was obtained, and diluting to 250 cc. The data indicate that the actual excess of sodium bicarbonate present in a cracker dough is much greater than appears from a determination of the hydrogen-ion concentration of the unaspirated dough suspension. In the data cited, the pH of the dough after the addition of the sodium bicarbonate was 7.20. After 10 minutes of aspiration it became 7.61, and after 20 minutes with continued aspiration became constant at 7.99.

TABLE X

ELECTROMETRIC TITRATION OF (A) LACTIC ACID AND (B) A FLOUR SUSPENSION RENDERED ACID WITH LACTIC ACID BY SODIUM BICARBONATE AND SODIUM HYDROXIDE

.020 N NaOH per 25 cc. .020 N lactic acid	pH	.020 N $NaHCO_3$ per 25 cc. .020 N lactic acid aspirated	pH	.020 N $NaHCO_3$ per 25 cc. .020 N lactic acid not aspirated	pH	.020 N NaOH per 25 cc. of suspension rendered acid to pH 3.31	pH
cc.		cc.		cc.		cc.	
0	2.77	0	2.77	0	2.77	0	3.31
5	3.28	5	3.26	5	3.27	5	3.65
10	3.63	10	3.62	10	3.60	10	3.97
15	3.99	15	3.96	15	3.95	15	4.29
20	4.41	20	4.38	20	....	20	4.82
21	4.51	21	4.48	21	....	21	4.95
22	4.70	22	4.65	22	....	22	5.22
23	4.93	23	4.87	23	4.80	23	5.53
24	5.38	24	5.28	24	....	24	5.93
25	6.25	25	6.12	25	5.22	25	6.39
26	7.30	26	7.08	26	5.46	26	6.78
27	9.85	27	8.23	27	5.66	27	7.13
28	....	..	....	..	....	28	7.54
29	10.19	..	....	..	....	29	8.06
30	10.51	..	....	..	....	30	8.35
				..	....	32	9.06
				..	....	34	9.80

The hydrogen-ion concentration of suspensions prepared from this dough before neutralization with bicarbonate had a hydrogen-ion concentration corresponding to a pH of 4.11. After the aspiration of this suspension there was only a slight decrease in hydrogen-ion concentration. It is probable that weak acids are present in the dough in such quantity that the carbon dioxide has little effect on hydrogen-ion concentration.

Aliquots from this dough suspension were then treated with standard sodium hydroxide and sodium bicarbonate solution. Fifty cc. of 0.02 normal sodium hydroxide added to 100 cc. of the acid dough suspension increased the pH to 9.08. Aspiration had no effect on the pH of this solution. Also 50 cc. of 0.02 normal sodium hydroxide solution was added to 10 cc. of the acid dough solution which had been aspirated. The pH of this solution was higher than that of the unaspirated solution treated in the same way. This indicates that the carbon dioxide present in the dough suspension affects the pH when titrated with sodium hydroxide, but the degree to which it is affected is relatively slight. In a similar manner, 50 cc. of standard sodium bicarbonate solution was added to the same solution. Before aspiration the suspension had a pH value of 6.53 and after aspiration it increased to 7.73. If the suspension to which the bicarbonate solution was added is aspirated before the addition of the bicarbonate, the increase in pH when it is aspirated again indicates that the carbon dioxide present in the suspension has a determinable effect on the hydrogen-ion concentration. Other data were secured which support this observation. It should be stated again, however, that suspensions prepared from doughs which produced the type of crackers sought by the industry have a pH of 7.00 to 7.10 and these suspensions on aspiration show that there is sufficient excess of sodium bicarbonate to give an alkalinity corresponding to a pH of approximately 7.8. As the data in Table XI show that even this pH is lower than would have been found had the carbon dioxide been removed before the addition of the sodium bicarbonate, it was deemed desirable to titrate to a greater alkalinity in order to compensate for this factor. The solutions of the sodium salts of the weak acids produced during fermentation will be more alkaline than 7.0, which justifies selecting a more alkaline endpoint in titration than absolute neutrality. Assuming that these acids are of the type of lactic acid, the neutralization point will be about pH 8.4, as indicated by the titration curve for lactic acid and sodium hydroxide presented in Figure 6. Phenolphthalein is accordingly available as an indicator in such titrations.

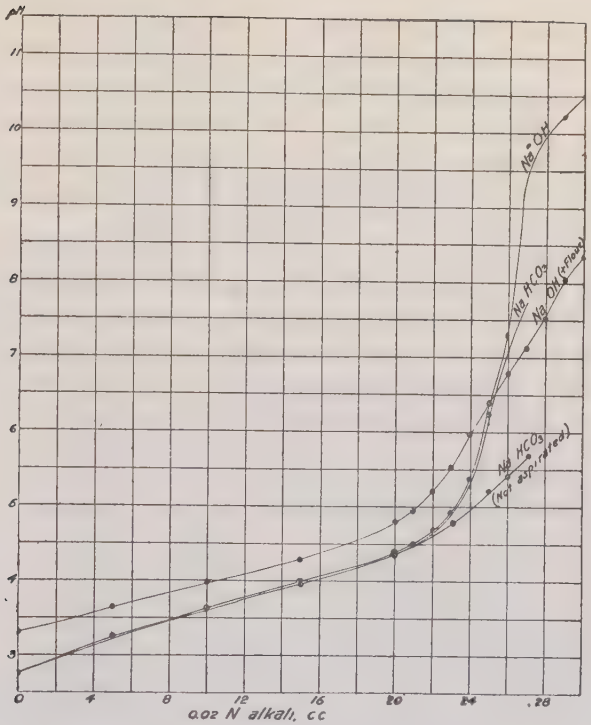

Fig. 6. Electrometric Titration Curves

(a) lactic acid with 0.02 N sodium hydroxide, (b) lactic acid with 0.02 N sodium bicarbonate, solution aspirated, (c) lactic acid with 0.02 N sodium bicarbonate solution unaspirated, (d) acidulated flour-water suspension with 0.02 N sodium hydroxide.

TABLE XI

EFFECT OF ASPIRATION ON THE HYDROGEN-ION CONCENTRATION (IN TERMS OF pH) OF CRACKER DOUGH SUSPENSIONS

No.	Treatment	pH
1	Dough after the addition of 8 lbs. $NaHCO_3$	7.20
2	The above suspension aspirated with H_2 for 10 minutes	7.61
3	The above suspension aspirated with H_2 for 30 minutes	7.99
4	Dough suspension before addition of $NaHCO_3$	4.11
5	Dough suspension before addition of $NaHCO_3$, after aspiration with H_2 for 30 minutes	4.16
6	No. 4 plus 50 cc. 0.20 N NaOH solution	9.08
7	No. 5 plus 50 cc. 0.20 N NaOH per 100 cc. of suspension	9.47
8	No. 7 aspirated for 30 minutes	9.45
9	No. 4 plus 50 cc. 0.20 N $NaHCO_3$ per 100 cc. of suspension	6.53
10	No. 5 plus 50 cc. 0.20 N $NaHCO_3$ per 100 cc. of suspension	6.68
11	No. 9 aspirated with H_2 for 30 minutes	7.73
12	No. 10 aspirated with H_2 for 30 minutes	8.15

Sodium hydroxide solutions must, for the reasons discussed, be used in titrating the acid cracker sponges. From the quantity

of standard alkali required to titrate a known weight of dough to a pink color with phenolphthalein, the quantity of sodium bicarbonate to be added to the dough can be calculated. There is also a distinct advantage in the use of a strong alkali such as sodium hydroxide. The pH change at the point of neutralization per increment of added alkali is greater than if a weaker alkali was used. This is demonstrated by the electrometric titration data presented graphically in Figures 5, 7, and 8.

In order to follow in more detail the changes which were taking place during the progressive stages of fermentation, electrometric titrations of dough suspensions in the direction of both increasing alkalinity and increasing acidity were made. The dough suspensions used in these studies were prepared in the same manner as described previously (32 grams of dough suspended in 250 cc. of water). Fiftieth normal acid and alkali were added to aliquots of this suspension in the ratio of 10, 20, 30, 40, or 50 cc. of the particular acid or alkali per 100 cc. of the suspension.

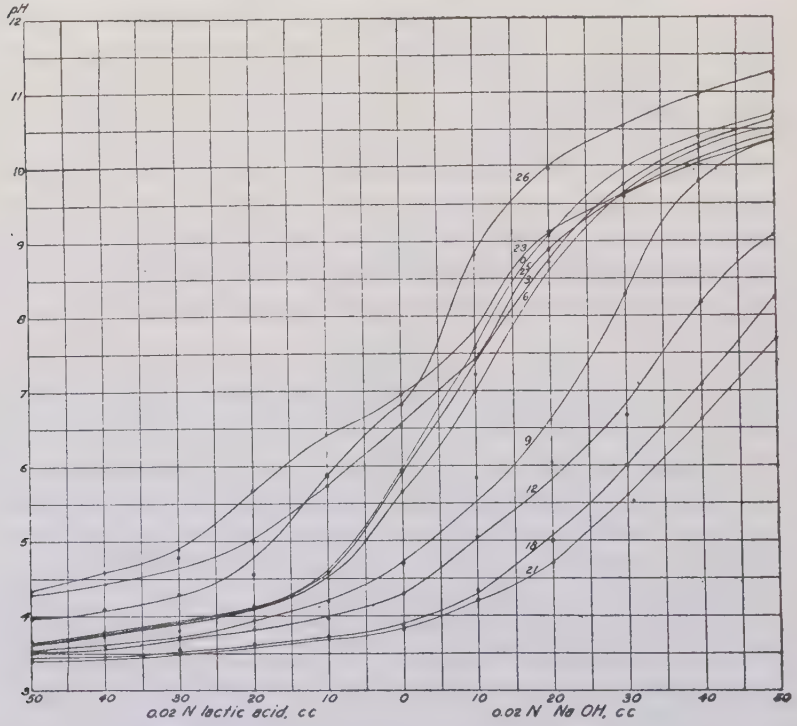

Fig. 7. Electrometric Titration Curves of Cracker Sponge Suspensions, of Cracker Dough Suspensions, and of Cracker Suspensions with 0.02 N Lactic Acid and Sodium Hydroxide Solution

The quantities of acid or alkali indicated were used per 100 cc. of the suspension. The numbers on the curves indicate the stage in the fermentation process, and refer to Table XII.

TABLE XII

HYDROGEN-ION CONCENTRATION (IN TERMS OF pH) ON THE ADDITION OF 0.02 N ACID AND ALKALI TO DOUGH SUSPENSIONS MADE AT VARIOUS STAGES OF THE FERMENTATION PROCESS

Stage of process		0.02 N lactic acid added per 100 cc. of dough suspension cc.						0.02 N NaOH added per 100 cc. of dough suspension cc.					Hydrogen-ion concentration of dough suspension	0.02 N NaOH required to bring suspension to pH 8.40
*Time	Treatment	50	40	30	20	10	0	10	20	30	40	50	CH x 10^{-5}	cc.
0	Fermentation	3.63	3.77	3.80	4.14	4.58	5.95	7.59	9.07	9.97	10.41	10.73	.112	14.75
2.5	"	3.62	3.73	3.90	4.14	4.56	5.93	7.22	8.72	9.77	10.31	10.65	.116	16.00
6	"	3.60	3.70	3.83	4.09	4.50	5.65	6.98	8.62	9.72	10.18	10.51	.226	17.25
9	"	3.55	3.62	3.73	3.89	4.18	4.68	5.83	7.20	8.77	9.82	10.35	2.080	30.50
12	"	3.50	3.57	3.68	3.82	3.96	4.28	5.05	6.04	6.66	7.08	9.03	5.280	42.00
18	"	3.41	3.48	3.55	3.62	3.72	3.86	4.33	5.00	6.00	7.00	8.22	13.550	54.00
21	"	3.40	3.43	3.48	3.58	3.69	3.82	4.21	4.70	5.61	6.63	7.67	15.100	60.00
23	$NaHCO_3$ added	4.34	4.58	4.87	5.66	6.42	6.95	7.81	9.11	9.60	10.11	10.45	.0113	10.50
25	Fermentation	4.23	4.43	4.67	5.00	5.73	6.54	7.42	8.89	9.82	10.18	10.37	.0287	16.00
26	Baked crackers	3.97	4.19	4.28	4.55	5.36	6.83	8.82	9.96	10.55	10.96	11.24	.0148	8.00

*The number on each line in Figure 7 indicates the stage in the fermentation process as shown in the above table, in terms of hours elapsed after mixing the sponge.

TABLE XIII

HYDROGEN-ION CONCENTRATION (IN TERMS OF pH) ON THE ADDITION OF 0.02 N ACID AND ALKALI TO DOUGH SUSPENSIONS MADE AT VARIOUS STAGES OF THE FERMENTATION PROCESS

Stage of process		0.02 N HCl added per 100 cc. of dough suspension						0.02 N NaOH added per 100 cc. of dough suspension					Hydrogen-ion concentration of dough suspension	0.02 N NaOH required to bring suspension to pH 8.40
*Time	Treatment	50	40	30	20	10	0	10	20	30	40	50	CH x 10^{-5}	cc.
0	Fermentation	2.79	3.08	3.55	3.96	4.80	5.73	7.88	9.33	10.23	10.48	10.58	.186	14.75
3	"	2.62	2.91	3.36	3.80	4.58	5.51	7.18	8.74	9.72	10.09	10.35	.308	18.00
6	"	2.42	2.87	3.18	3.53	4.38	5.41	7.08	8.67	9.65	10.07	10.31	.390	18.50
9	"	2.45	2.72	3.01	3.48	4.07	5.12	6.58	7.89	9.36	10.01	10.26	.754	21.50
12	"	2.49	2.62	2.84	3.24	3.84	4.60	5.76	7.10	8.60	9.67	10.16	2.520	28.25
18	"	2.45	2.60	2.86	3.18	3.58	4.08	4.77	5.90	6.96	8.00	9.25	8.760	42.50
21	"	2.42	2.55	2.79	3.12	3.55	3.99	4.60	5.54	6.69	7.61	8.99	10.200	45.50
24.5	"	2.45	2.62	2.79	3.11	3.52	3.92	4.45	5.31	6.52	7.18	8.18	12.000	52.50
26.5	$NaHCO_3$ added	4.97	5.44	6.15	6.61	7.03	7.44	8.10	9.01	9.52	9.94	10.01	.00364	8.00
29	Additional fermentation	4.24	4.43	5.56	6.12	6.78	7.20	8.52	9.28	9.79	10.08	10.13	.00628	9.00
30	Baked cracker	2.99	3.33	3.75	4.43	5.56	8.52	9.86	10.50	10.89	10.85	11.06	.000302	—.75

*The number on each line in Figure 8 indicates the stage in the fermentation process as shown in the above table, in terms of hours elapsed after mixing the sponge.

The hydrogen-ion concentration of the suspensions was then determined electrometrically. The results obtained when lactic acid and sodium hydroxide were used are given in Table XII, while the results with hydrochloric acid and sodium hydroxide are given in Table XIII. The hydrogen-ion concentration of the suspension before the addition of acid or alkali and the quantity of standard alkali required to bring the suspension to the pH 8.40 are also given. From this latter value the quantity of sodium bicarbonate required to neutralize the acids of fermentation can be calculated. Figures 7 and 8 show the data from these two tables in graphic form.

The data in these two tables again show the rapid rate at which the hydrogen-ion concentration increased between the 6th

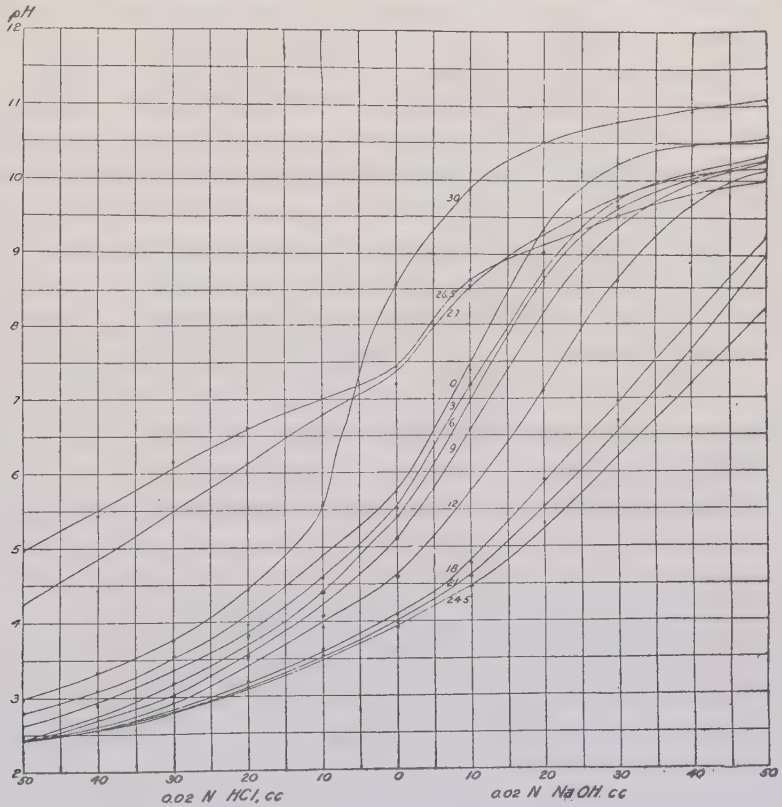

Fig. 8. Electrometric Titration Curves of Cracker Sponge Suspensions, of Cracker Dough Suspensions, and of Cracker Suspensions with 0.02 N Hydrochloric Acid and Sodium Hydroxide Solution

The quantities of acid or alkali indicated were used per 100 cc. of this suspension. The numbers on the curves indicate the stage in the fermentation process, and refer to Table XIII.

and 12th hours of the fermentation period. It was also during this period that most of the titratable acidity was developed.

During the later stages of fermentation the rate of acid production decreased, as indicated by both hydrogen-ion cencentration and titratable acidity data. From these data the quantity of sodium bicarbonate required to bring a dough, prepared in the manner which has been described, to the proper hydrogen-ion concentration could be calculated. In making this calculation sufficient bicarbonate must also be included to bring to a pH of 8.4 the 112 pounds of fresh flour which is added when the dough is made. In Figure 9 the pounds of sodium bicarbonate required to neutralize the acidity of a 5-barrel dough can be read off directly. On the axis of abscissa is plotted the 0.02 normal sodium hydroxide in cc. required to bring 100 cc. of the extract (that is, 12.8 grams of the dough) to the proper hydrogen-ion concentration. On the axis of ordinates is plotted the corresponding quantity of sodium bicarbonate required per 5-barrel dough. From the following equations such a graph can be drawn for any fermentation process in which the standard alkali used is of the same normality.

$$1)\ \frac{\text{Pounds of dough used in sponge} \times .00168 \times \text{cc. 0.02 N NaOH}}{\text{Grams of dough for the test}} =$$

pounds of sodium bicarbonate required for neutralization of sponge.

$$2)\ \frac{\text{Pounds of flour added} \times .00168 \times \text{cc. 0.02 N NaOH}}{\text{Grams of flour used in test}} =$$

pounds of sodium bicarbonate required to bring added flour to required pH

For any controlled process in which a single flour is used, the value determined by equation 2 is a constant and need be calculated only once.

Basing our calculations on a 1200-pound sponge, which would require 40 cc. of 0.02 normal sodium hydroxide for neutralization of 12.8 grams of dough, we obtain a sodium bicarbonate equivalent of 6.28 pounds from equation 1. Assuming that 112 pounds of flour was added in the preparation of the dough, and that 4 cc. of 0.02 normal alkali was required to bring 10 grams of flour in suspension to pH 8.4, we find from equation 2 that 0.752 pounds of bicarbonate is required. This means that such a dough should require 7.03 pounds of sodium bicarbonate to effect the neutralization of the acids of fermentation and bring the added flour to the same alkalinity.

In addition to requiring sodium bicarbonate sufficient merely to neutralize the acids of fermentation, it was indicated in another section that for definite reasons a certain excess of bicarbonate was desirable. This excess will vary, depending upon the type of cracker desired in any particular region. In the factory in which part of this work was conducted, an excess of 1.5 pounds of sodium bicarbonate over that required to neutralize the acids of fermentation and the acids in the flour, as calculated through the use of equations 1 and 2, gave satisfactory results. Hence for the particular dough under discussion 8.5 pounds of sodium bicarbonate would be the correct quantity to use in mixing the dough.

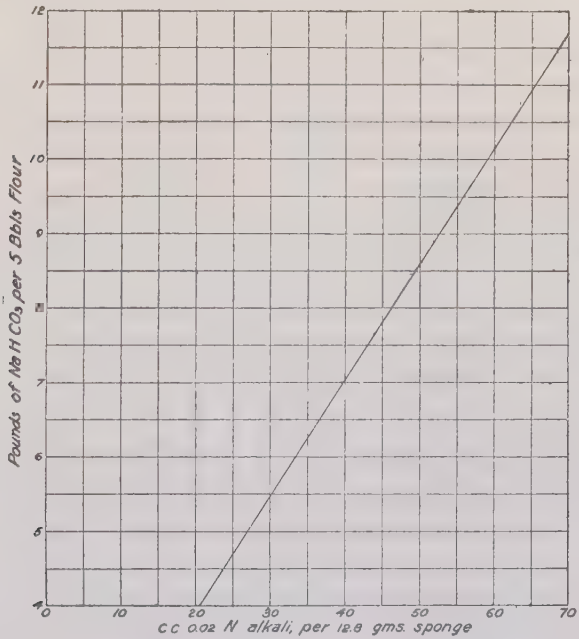

Fig. 9. Graph for Converting Standard Sodium Hydroxide Solution Used to Neutralize the Acids of Fermentation and the Acidity of the Added Flour to Pounds of Sodium Bicarbonate to be Added to a Five-Barrel Dough

Using the graph shown in Figure 9, the quantity of bicarbonate required was determined and added to a number of doughs in actual plant practice. The crackers produced from such chemically controlled doughs were invariably of the type desired, having the proper degree of brownness, specific volume, and eating qualities. The last property is largely a matter of the effect of hydrogen-ion concentration on the properties of the proteins. This method of chemical control has the advantage over the judgment of the experienced man in that if carried out properly it always gives correct results, while judgment of

persons, no matter how experienced, is never infallible. In a series of observations it was found that satisfactory crackers were produced when the quantity of bicarbonate used was equivalent to that indicated as required by titration. This is shown by the data in Table XIV and Figure 10. The odd numbers refer to crackers which the foreman considered satisfactory; and in these cases, with the exception of sample No. 1, the quantity of soda to be added was calculated according to the formulas which have been given. It will be noted that the volumes occupied by 10 crackers of the same type, as 1, 3, 5, or 7, 9, and 11, are comparatively uniform.

The even numbers refer to crackers in which there was either too much or too little soda as indicated in Table XIV, the height of the pile of 10 crackers being a convenient basis of comparison. The crackers which had too little or no excess of sodium bicarbonate occupy less volume because there is a limited quantity of carbon dioxide liberated to expand the dough in baking. On the other hand, when there is too great an excess of bicarbonate present, the crackers are expanded too much.

Just as important as the volume of the crackers is the color, which, of course, can not be shown in the photograph. The acid crackers, however, all lacked the bloom which is so desirable. A marked excess of alkali results in the production of crackers which are too dark colored. The crackers indicated as satisfactory, had what is termed in the industry "bloom."

It is to be noted that the desirable crackers were produced from doughs which had pH values of approximately 7 immediately after the sodium bicarbonate had been added. In such doughs the fall in pH during the following three hours before it was baked into crackers was relatively slight. If the pH after the addition of the bicarbonate was as low as 6.80, the increase in acidity during the next three hours entirely neutralized any excess of bicarbonate which had probably been present at the beginning, and acid crackers resulted. The same observations have been noted in a foregoing section.

Aside from their practical significance, the data presented in Tables XII and XIII and Figures 7 and 8 are of scientific interest. The effect of the flour substances causing the titration curves to flatten out or to approach a certain maximum is indicated both on the acid and the alkaline side of neutrality. The curves on the acid side of neutrality tend to converge and reach a maximum at a pH equivalent to a higher hydrogen-ion concentration when the titration is conducted with hydrochloric acid than with lactic acid. Another point of interest is the buffer effect of the sodium bicarbonate which was added to the dough. Altho the pH in the freshly mixed dough was

TABLE XIV

DATA ON THE CONTROL OF CRACKER DOUGH ACIDITY BY DETERMINATION OF THE QUANTITY OF SODIUM BICARBONATE TO BE ADDED

No.*	$NaHCO_3$ actually added Lbs. per 5-bbl.	$NaHCO_3$ calculated Lbs. per 5-bbl.	Notes on crackers	pH immediately after addition of $NaHCO_3$	pH just before baking	pH of crackers
1	8.50	8.75	Satisfactory	7.07	6.88	7.39
2	7.50	9.00	Too little $NaHCO_3$	6.71	6.29	6.37
3	9.75	9.75	Satisfactory	6.98	6.83	7.34
4	7.00	....	Too little $NaHCO_3$	6.73	6.04	5.95
5	7.75	7.75	Satisfactory	7.05	6.86	7.18
6	9.50	....	Too much $NaHCO_3$	7.51	7.33	9.18
7	9.50	9.50	Satisfactory	7.22	6.90	7.74
8	9.00	....	Too little $NaHCO_3$	6.80	5.95	5.95
9	9.75	9.75	Satisfactory	6.97	6.82	7.12
10	8.00	....	Too little $NaHCO_3$	6.88	6.17	6.29
11	9.50	9.50	Satisfactory	7.00	6.79	7.22
12	9.50	....	Too much $NaHCO_3$	7.49	7.40	10.01
13	8.00	8.00	Satisfactory	7.05	6.99	7.89
14	8.50	....	Too little $NaHCO_3$	6.75	6.05	6.11

*The numbers refer to the crackers shown in Figure 10.

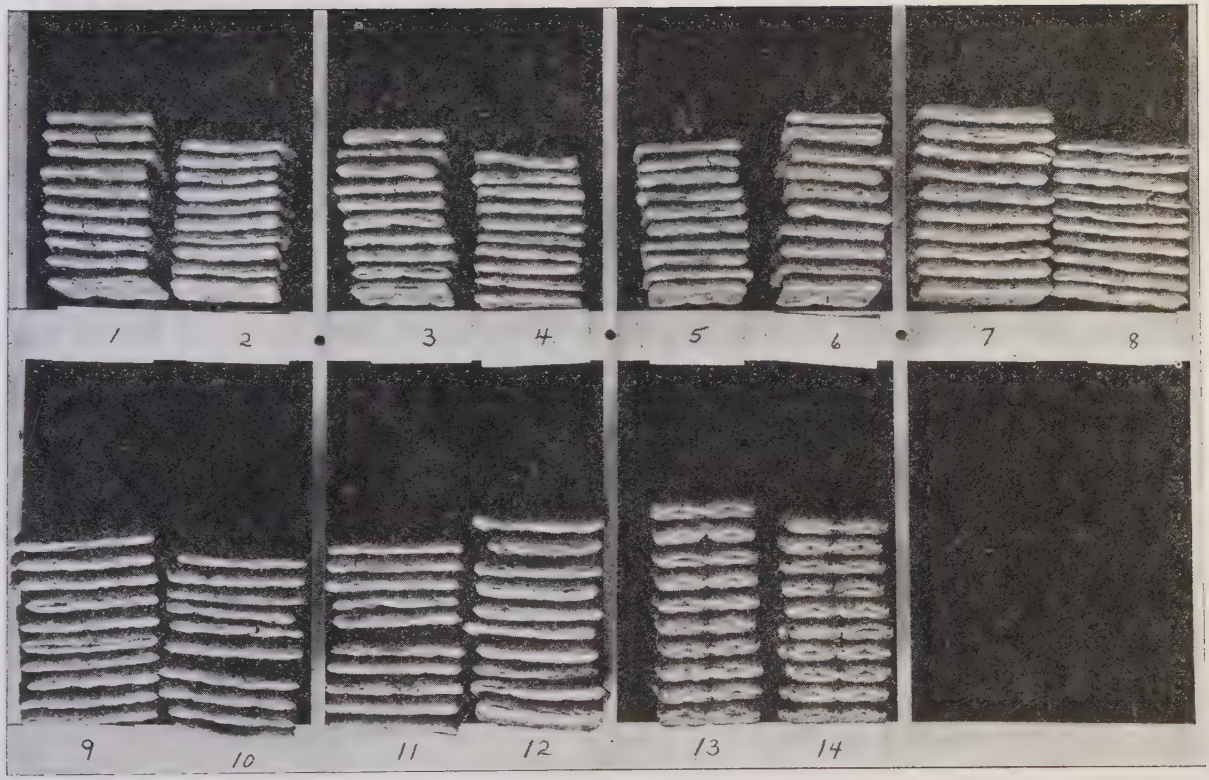

Fig. 10. Comparative Volumes of Crackers Baked from Various Doughs

The odd numbered samples were baked from doughs containing the proper quantity of sodium bicarbonate. Samples 2, 4, 8, 10, and 14 were baked from doughs containing too little sodium bicarbonate. Samples 6 and 12 were baked from doughs containing too much sodium bicarbonate. For a description of the treatment, see Table XIV.

distinctly higher than that of the original flour, the addition of 50 cc. of 0.02 normal sodium hydroxide to 100 cc. of the dough suspension only increased the pH of the suspension containing bicarbonate from 7.44 to 10.01, while the increase in the pH of the original dough suspension on the addition of the same quantity of sodium hydroxide was from 5.73 to 10.58. That this buffering effect was due to sodium bicarbonate and not to substances formed by neutralization of the acids of fermentation is shown by the fact that during the 3-hour period immediately after the addition of the bicarbonate the dough became more acid (hence some of the bicarbonate must have disappeared), but nevertheless the addition of 50 cc. of 0.02 normal sodium hydroxide solution to a unit quantity of suspension increased the pH from 7.20 to 10.13. The titration curve of the baked cracker suspension is of interest in this connection. Baking has removed from the cracker other buffers besides the bicarbonate, hence we get the curves through the wide range of pH shown in these figures.

Proteolysis in cracker dough fermentation.—The purpose of the long fermentation period in cracker doughs is not only to increase the acidity, but more particularly to allow the gluten proteins to undergo certain changes. Whether the significant changes which take place are colloidal or physical, as distinguished from purely chemical changes, has not been known. If physical or colloidal alone, can the results be obtained in some way other than by fermentation? If chemical alone, can the desired results be produced by deliberately inducing chemical changes? These are some of the problems which must be solved before we will know the mechanism by which the tough gluten is rendered capable of producing the type of tender, crisp crackers desired.

Before undertaking a study of the changes in gluten properties which occur during the fermentation process, it may be well to consider the agencies which effect the changes. It is known that changes in hydrogen-ion concentration may influence the properties of gluten to a remarkable degree. The proteases present in the flour, and those in the yeast also, act on the flour proteins and if proper conditions prevail will split them into their component amino acids. In view of the significance of proteases in flour and in yeast, the literature bearing on this phase of the subject will be briefly reviewed.

Buscalioni and Fermi (1898) were among the early workers who presented experimental evidence which indicated the presence of proteolytic enzymes in plants. Liquefaction of gelatin was used as a criterion of proteolytic activity. Garup and Besanez (1874-1875) investigated the enzymes of barley, hemp, and vetch, and expressed the belief that enzymes of the pepsin type were present. Altho

greatest activity was shown in a .2 per cent hydrochloric medium, they did not designate the enzyme as pepsin but a "peptonbildenes" ferment. This work led to the idea of peptic digestion in plants. It was demonstrated that in certain instances the amino acids, leucin, tyrosine, and tryptophan were found. The idea that such enzyme action resembled tryptic digestion in animals was suggested, but as the ferment showed its greatest activity in slightly acid medium, these two types of digestion are not entirely comparable. This theory of plant protein proteolysis was known as the plant trypsin theory.

It is at this stage of knowledge of proteases that Vines (1902-09) began to publish, and we owe much of our knowledge of these plant enzymes to him. In his papers, he discusses work done on a wide variety of materials, both seeds and plant tissues, but as our interest is concerned chiefly with the cereal grains, the discussion will be limited largely to them.

In his earlier work, Vines held to the trypsin theory of plant protein digestion. As early as 1902, however, he casts some doubt on this theory as the mechanism by which proteins were converted into assimilable products. Certain of his experiments indicated that the optimum hydrogen-ion concentration for barley proteolytic ferments is on the acid side of neutrality. Autolyzed extracts of wheat germ produced results similar to those given above. Further work led Vines to ascribe plant protein digestion to the activity of two enzymes: a protease, peptic in nature, capable of digesting protein to peptone but not acting on these whether produced by it or added as Witte-peptone; and a protease, ereptic in nature, incapable of digesting protein but capable of digesting the products of peptic digestion or added Witte-peptone. Vines (1906) summarizes his work on proteases in seeds with the following statement: "The ungerminated seeds of plants were found to contain a protease which acted (a) immediately on Witte-peptone and (b) more or less slowly on the reserve proteids of seeds. The germinated seeds were found to contain a protease that digested fibrin: this protease was also found in certain cases to be slowly developed in the substance of ungerminated seed in the course of the experiment." Hence during the first few days of germination, the course of proteolysis is confined to the action of the erepsin-like enzyme on the digestible reserve proteins. Then a pepsin-like ferment is developed which supplies additional material upon which the ereptases can act. Evidence confirming the conclusions reached by Vines was presented by Dean (1905).

In the light of Vines' work, Ford and Guthrie (1908) made observations on flours. They noted the deleterious effects of even small quantities of proteolytic ferments on gluten, and stated that this

deleterious effect manifested itself in the inability of the dough to retain the gases formed during fermentation. When active proteases were deliberately added to bread doughs, such doughs failed to rise and were characterized by what was termed "rotten" gluten. They carried on certain experimental work to determine whether protease activity in a flour could be the reason for "weakness" in certain flours. They suspended 5 grams of flour in 50 cc. of 1.5 per cent gelatin solution and noted whether the gelatin was liquified. In two cases in a series of 12 flours liquefaction did occur, and these particular flours were known to produce bread of poor quality. They ascribe the "weakness" of these flours to the effect of peptic enzymes in breaking down the gluten.

Baker and Hulton (1908) conducted experiments confirming those of Ford and Guthrie. Small quantities of flour were digested with water saturated with chloroform for 20 hours at 37°C. and at the end of this time tested for protease activity by means of the Hopkins and Cole reaction. Of the nine flours which they used only one gave a strong reaction for trytophane. When Witte-peptone was added to the mixture being digested, a strong tryptophane reaction was given by all the flour under observation. These experiments taken in conjunction with the observations of Vines seem to indicate that most flours might be expected to contain ereptic enzymes. On the other hand, in only a relatively small number of the flours studied was the presence of peptic enzymes demonstrated. When peptic enzymes were present the bread baked from such flours showed the deleterious effects of the enzyme on the gluten.

More recently Swanson and Tague (1916) subjected flour suspensions to auto-digestion and determined the increase in amino nitrogen at certain intervals by means of the formol titration of Sorensen (1912). These workers found that the presence of salts and organic substances affected the rate at which amino acids were produced.

Stockham (1920) employed the method of Buscalioni and Fermi (1898) in determining the proteolytic activity of certain flour extracts. A 1.5 per cent gelatin media saturated with nitro-benzine was prepared. The time required for extracts from different flours to bring about liquefaction of the gelatin was used as an index of the proteolytic activity of the flour. Data were presented to show that in general, the lower the grade of flour, the more rapid the liquefaction of the gelatin. It was regarded as possible that the lower grade flours contain portions of the scutellum, which organ according to the work of Mann and Harlan (1915) possesses proteolytic enzymes which are responsible for liquefying the gelatin. Stockham cut wheat so as to separate the blossom end from the germ end, and milled flour from

the cut wheat berries. Extracts prepared from flour milled from the germ end segments liquefied gelatin in 330 hours, while it required 720 hours for extracts prepared from the blossom end flour to effect this result. Hence it is likely that it is the enzymes supplied by the scutellum of the germ which caused the liquefaction. That such proteolytic activity had deleterious effects on the flour may be seen from the fact that the loaf volume of bread made from flour milled from blossom ends was 2700 cc. while that of flour prepared from wheat ends containing the embryo was only 2050 cc. The texture and expansion (loaf volume minus dough volume) of the smaller loaf were also decidedly inferior to those of the larger loaf. Stockham also noted a decrease in proteolytic activity in flours which had been stored for any length of time.

Sharp and Elmer (1924) stated that it is conceivable that the enzymes of flour would not digest foreign proteins at the same rate as the native proteins of flour, and as it is the flour proteins in which we are mainly interested such proteins should be used as sub-strates. They allowed flour suspensions preserved with toluene to undergo auto-digestion and then determined changes in distribution of nitrogen in the fractions soluble in 5 per cent potassium sulphate solution, 70 per cent alcohol, and nitrogen not accounted for in these two fractions. It was concluded that the proteolytic enzymes of flour were capable of digesting flour protein if given sufficient time in which to act. They found that the fraction of protein soluble in 5 per cent potassium sulphate solution increased with time, largely at the expense of the alcohol soluble protein. There appeared to be some increase of protein in the gliadin fraction.

That proteolytic activity of the flour may be a significant factor in cracker dough fermentation is clear when one considers the 24-hour fermentation period to which such doughs are ordinarily subjected.

It is probable, however, that the proteases added with the yeast have a greater proteoclastic effect on the flour-proteins than those present in the flour.

Hahn (1898), Geret and Hahn (1898), state that yeast contains a powerful digestive enzyme of the nature of trypsin. This enzyme, just as those responsible for fermentation, is present only within the yeast cell. Hahn prepared a solution free from yeast cells which showed considerable proteolytic activity. On the basis of his work, the yeast proteoclastic enzyme has received the name, endotryptase. Hahn also measured the activity of the enzyme in terms of the time required to liquefy gelatin. The time required for complete liquefaction of gelatin was two or three days, altho at the end of one day the gelatin showed evidence of liquefaction. Vines (1909) be-

lieved that the proteoclastic enzymes of yeast are contained within the cell, but that such enzymes are not of the trypsin type. He states that the proteoclastic enzymes of yeast consist of a peptic and an ereptic enzyme, the combined action of which gives the results of tryptic digestion. This is the same conclusion that Vines reached regarding such enzymes in the higher plants.

Dernby (1917) presents evidence to show that there are three types of proteoclastic enzymes in yeast, an endopeptase and an endoereptase, which have essentially the properties ascribed to them by Vines, and an endotryptase incapable of attacking yeast protein but capable of attacking albumen, gelatin, and caseinogen, and converting them into peptides and amino acids.

Thus far the discussion has been limited to a consideration of the presence of proteoclastic enzymes within the cell. A question arises concerning the presence of extra-cellular proteases in yeast suspensions, in regard to which there are no definite conclusions. Hansen (1888), Koehler (1892), and Lindner (1891) state that yeast effects the liquefaction of gelatin. On the other hand, Wehmer (1895) could ascribe no liquefying action to normal yeast. Hahn and Geret (1900) believe that any proteoclastic action which occurs is due to enzymes liberated from dead cells. The latter view seems to be the one most generally accepted.

In investigating the changes which enzymes or other agencies effect during cracker dough fermentation, both physical and chemical methods may be used. Efforts were first directed toward determining such changes by purely chemical methods.

It is a well known fact that certain proteolytic enzymes, if given sufficient time, will split the proteins to the amino acid stage. Hence, if proteolytic activity be vigorous enough in the fermenting dough, we should expect to find amino acids in the dough solution as one of the cleavage products derived from the proteins. Osborne (1916), and Blish (1918) have used copper hydroxide as a reagent for precipitating proteins in solution as a first stage in the determination of non-protein nitrogen. Blish (1918) presents data to show that copper hydroxide precipitates proteins and split products down to the peptide and amino acid stage. Another well known protein precipitant is stannous chloride. The use of this reagent was first described by Scherning (1897). It has since been used by both Sorenson (1909) and Dernby (1917). The technique of its use has recently been greatly improved by Olsen (1923). Stannous chloride has been found to precipitate somewhat less of the split products of proteolysis than does copper hydroxide. These two agents were used in essentially the manner described by Olsen (1923), hence his methods will be given somewhat in detail

The stannous chloride was prepared after the method of Scherning (1897). Fifty grams of tin was dissolved in sufficient strong hydrochloric acid to which a few drops of platinic chloride has been added to catalyze the reaction. When solution was complete, it was evaporated almost to dryness and then diluted to 500 cc. Six cc. of this reagent was used to precipitate the proteins from suspensions. The suspensions which were used were prepared in the following manner: One hundred grams of dough was shaken until a smooth suspension was obtained and the volume made up to 500 cc. in a volumetric flask. To 50 cc. of this suspension was added the 6 cc. of stannous chloride solution. It was found that precipitation of the proteins was most perfect when the pH of the solution was approximately 5.9, hence the suspension was brought to this pH with sodium hydroxide using bromcresol-purple (Clark, 1922) as an indicator. At this pH the indicator changes from yellow to a faint blue. The solution was then made up to 100 cc., centrifuged, filtered, and 75 cc. of the filtered liquid (representing 5.43 grams of flour) taken for the determination of nitrogen by the Kjeldahl method.

Fifty cc. of the same dough suspension was taken and the precipitation carried out by means of copper hydroxide in the following manner: To the 50 cc. of dough suspension was added 20 cc. of N/5 sodium hydroxide. Sufficient N/5 copper sulfate was added, the correct quantity of which was noted by the method suggested by Olsen. A few drops of phenolphthalein were placed in the solution. Upon addition of copper sulfate the color changes from purple to blue to green. The change from blue to green takes place when the proper quantity of sodium hydroxide has been added. These solutions were made up to a volume of 100 cc., centrifuged and filtered, and 75 cc. of the solution was taken for the nitrogen determination. It was frequently found that even when the utmost care was exercised it was impossible to obtain water-clear solutions by one precipitation. In such cases two precipitations were made.

In Table XV are recorded the results which were obtained when the above precipitations were carried out on cracker doughs. The non-precipitable nitrogen has been calculated to a basis of 100 grams of the flour in the dough suspension. The data indicate that without question proteolysis does occur during the fermentation of the cracker sponge. At the end of an 18-hour period, at which time the bicarbonate and fresh flour are added to the sponge, the protein non-precipitable with tin had more than doubled and the protein non-precipitable with copper showed a significant increase. A portion of the unneutralized dough was set aside and fermentation allowed to continue for 24 hours longer. Here again the nitrogen non-precipitable by these two reagents

indicated that proteolysis had taken place. These data indicate nothing concerning the organism causing, or the mechanism of, the proteolysis which occurs. It may be due to enzymes present in the flour, enzymes added with the yeast, micro-organisms present in the flour and yeast, or conditions induced in the fermenting dough by the organisms present. Certain of these factors will be discussed in following sections.

TABLE XV

PROTEOLYSIS IN A CRACKER DOUGH DURING THE FERMENTATION PERIOD AS INDICATED BY INCREASE IN NITROGEN NON-PRECIPITABLE BY SN AND CU

Time	Nitrogen per 100 gms. of cracker flour	
	Sn—non precipitable N	Cu—non precipitable N
Hours	mg.	mg.
0	43.27	23.02
5	79.74	26.70
11	83.79	28.36
18	100.92	36.46
24	122.10	37.75
48	264.27	91.34

Effect of hydrogen-ion concentration on dispersion of flour proteins.—In certain of the determinations discussed in the preceding section, considerable difficulty was experienced in bringing about complete clarification of the suspension. Clarification was especially difficult in the case of suspensions prepared from the cracker sponge in the later stages of fermentation before the addition of the sodium bicarbonate. When such suspensions were centrifuged in order to remove as much of the suspended matter as possible, only a small fraction of the protein material was thrown down.

Gortner and Sharp (1923) noted the effect of hydrogen-ion concentration on the imbibitional capacity of the flour proteins. They found greatest swelling, as determined by viscosity methods, to occur in an acid medium at a pH of approximately 3.0. In cracker dough fermentation, this pH is never attained. The acids of fermentation do, however, bring about a hydrogen-ion concentration corresponding to a pH of 3.8 to 4.2. It is obvious that a swelling and dispersion of the proteins must occur, making it impossible to remove them from suspension by centrifuging.

It was suggested that this acidity alone might perhaps be a factor which contributed to the "conditioning" of the gluten. The changes in degree of dispersion of the proteins were accordingly studied at various stages of the fermentation period in relation to both time and hydrogen-ion concentration. Table XVI gives the experimental data obtained and the results are expressed graphically in Figures 11 and 12.

The suspensions which were used were prepared as described in some of the previous work, 32 grams of dough being disintegrated by shaking the particles suspended in 250 cc. of distilled water. These suspensions were then centrifuged. Whenever possible the supernatant

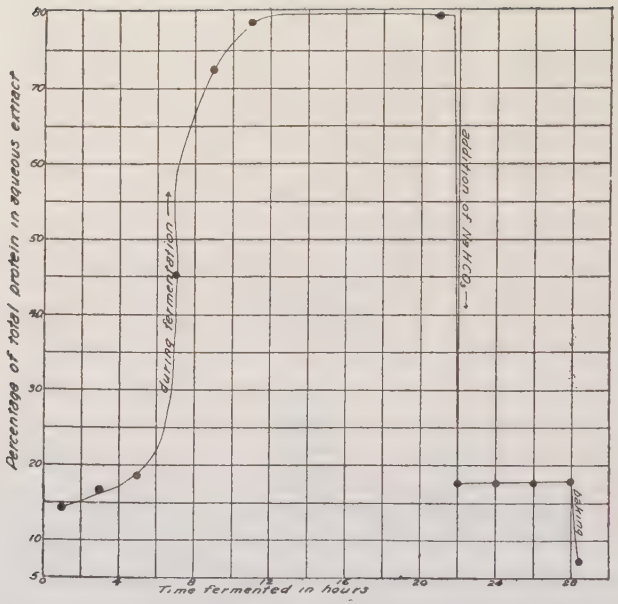

Fig. 11. Changes in Percentage of Total Protein in Aqueous Extract Determined During Various Stages of Cracker Manufacture

(a) During fermentation of the sponge, (b) immediately after neutralization of the acid sponge with sodium bicarbonate, (c) during subsequent fermentation of the neutralized dough, (d) during the baking process.

liquid was filtered, but it was found that this could not be done after the first 6 to 8 hours of fermentation, as the pores of the filter soon became stopped and only a few cubic centimeters of liquid could be obtained. In these cases aliquots of 25 cc. of the solution had to be taken without filtration for the nitrogen determination.

A study of Table XVI and the accompanying graphs shows that during the first 5 to 8 hours of the fermentation period, when the hydrogen-ion concentration changed at a relatively slow rate, there was only a slight increase in the percentage of the total protein dispersed. With more rapid increase in hydrogen-ion concentration the increase in percentage of dispersed protein was enormous. In the series of August 17, the pH changes, 5.51 to 5.24 to 4.83 increased the fraction of total protein in a dispersed condition from 18.50 to 45.28 to 72.54 per cent respectively. With further increase in hydrogen-ion concentration there was only a slight increase in protein non-precipitable by centrifuging.

That the greater part of this increase in protein dispersed in the liquid phase is due to increased hydrogen-ion concentration and to no other factor, is indicated by the fact that when the sodium bicarbonate is added and the pH brought to approximately 7, the dispersed protein is reduced to almost its original value. That it does not return to its

original value may be ascribed to two factors. In the previous section it was shown that proteolysis occurred during fermentation and such proteolysis would tend to increase the quantity of nitrogen not precipitated by the use of the centrifuge. Sodium chloride is, moreover, added during the final doughing up process. Osborne (1907) has shown that dilute solutions of neutral salts will extract the vegetable globulins, hence the added salt may operate to dissolve a part of the globulin content of the flour. For these reasons it might be anticipated that more dispersed protein would be found in the remixed dough than in the freshly mixed sponge. When the dough was baked into crackers there was a decrease in dispersed protein (with calculations based on a unit of dough), due, no doubt, to the coagulation of heat-coagulable proteins.

Further evidence that the dispersion of the proteins is a function of hydrogen-ion concentration is found in the data accumulated on August 17. A portion of the neutralized dough which had been fermented for 22 hours was allowed to ferment for 38 hours more, a total period of 60 hours. At the 21-hour period the pH of the original sponge was 3.92 and the protein in suspension 79.68 per cent. The addition of bicarbonate brought the pH to 7.81 and reduced the protein in suspension to 17.58 per cent. Continuing the fermentation for 13 hours brought the pH to 4.60 and the dispersed protein increased to 65.12 per cent. After 60 hours fermentation, or 38 hours after the bicarbonate addition and remixing, the pH was 4.09 and about the same percentage of the protein was dispersed as before the addition of the sodium bicarbonate. The effect of increasing the hydrogen-ion concentration during this second fermentation period is shown by the dotted line and the open circles of Figure 12. At this 60-hour period there was also a notable increase in nitrogen non-precipitable with tin and copper.

Further evidence of the effect of hydrogen-ion concentration on protein suspensions is indicated by the following data. A flour-water suspension was prepared and 2 cc. of normal lactic acid added. The addition of the lactic acid brought the pH to 3.26. This suspension was centrifuged in the hand centrifuge, the nitrogen content of the centrifugate was determined and found to be 88.98 per cent of the total nitrogen.

The data which have been presented indicate in no uncertain manner the cycle of changes which the gluten proteins undergo during the various stages of fermentation. The next question which arises is, are these changes (those due to hydrogen-ion concentration) only incidental to fermentation or proteolysis, or does increased acidity play a part in conditioning the gluten? The following section will attempt

to differentiate changes which might be ascribed to acidity from those which might be ascribed to proteolysis.

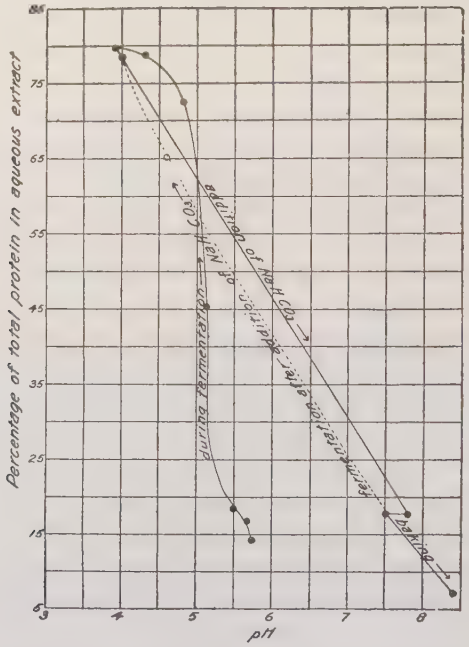

Fig. 12. Effect of Hydrogen-ion Concentration on Percentage of Total Protein in Aqueous Extracts

These were prepared from (a) an acid cracker sponge during fermentation, (b) the neutralized dough after the addition of sodium bicarbonate. The percentage of the total protein in the aqueous extract of the cracker after baking is also shown.

TABLE XVI

PROTEIN (N x 5.7) IN A DISPERSED STATE AT VARIOUS STAGES IN THE PROCESS OF CRACKER MANUFACTURE*

July 25, 1923

Time	Treatment	Dispersed protein based on bour	Dispersed protein based on protein in flour
Hrs.		%	%
0		1.25	14.44
2.5	Fermentation	1.47	15.74
4.5	"	1.59	17.04
6.5	"	1.72	18.38
9.0	"	2.09	22.30
11.0	"	3.64	38.98
13.0	"	4.13	44.24
18.0	"	4.49	48.04
20.0	"	7.80	83.46
21.0	$NaHCO_3$ added	1.63	19.64
24.0	Fermentation	1.81	19.32
24.0	Baked crackers	.79	7.40

TABLE XVI—*Continued*

Time	Treatment		Dispersed protein based on bour	Dispersed protein based on protein in flour
Hrs.		August 17, 1923	%	%
1	Fermentation	5.77	1.33	14.28
3	"	5.68	1.56	16.66
5	"	5.51	1.73	18.50
7	"	5.24	4.23	45.28
9	"	4.83	6.79	72.54
11	"	4.33	7.37	78.86
21	"	3.92	7.45	79.68
22	$NaHCO_3$ added	7.81	1.64	17.58
24	Fermentation	7.67	1.64	17.58
26	"	7.59	1.64	17.58
28	"	7.52	1.67	17.86
35	"	4.60	6.09	65.12
60	"	4.09	7.34	78.48
Crackers	Baked crackers	8.40		7.20
		August 24, 1923		
0		5.95	1.42	15.14
2	Fermentation	....	1.66	17.70
4	"	....	1.93	20.60
6	"	5.49	2.97	31.76
8	"	5.12	7.27	77.72
10	"	4.60	8.17	87.42
12	"	4.26	8.39	89.52
16	"	3.92	8.62	92.14
18.5	"	3.86	8.78	93.70
20	$NaHCO_3$ added	7.17	1.85	19.62
23	Fermentation	6.91	1.83	19.58
24	Baked crackers	7.23	.698	5.46

*Total protein (N x 5.7) in the flour was 9.35%.

Differentiation of changes produced by proteolysis from those produced by hydrogen-ion concentration.—The work described in the preceding section suggested that perhaps the increase in hydrogen-ion concentration was sufficient in itself to bring about the proper conditioning of the gluten. A method was, therefore, sought for measuring the effects of fermentation and increased hydrogen-ion concentration on certain physical properties of the gluten. Bailey and LeVesconte (1924) had found the Chopin (1921) extensimeter useful for such purposes and hence its application to such studies as these was suggested. Their paper describes the instrument, and the manner in which it is used. It is sufficient to state that a sheet of dough of uniform dimensions is stretched until it ruptures, at which time its area is measured. This affords a measure of the extensibility of the dough, a physical property of considerable importance.

As cracker doughs of the type under observation contain so little water as to be too stiff for use with the Chopin apparatus, about 600 grams of the dough was taken from the trough and mixed in a small mixer with sufficient water to give a dough of the desired consistency. Also sufficient 25 per cent sodium chloride solution was added that the total water added would contain the equivalent of 2 per cent sodium chloride. It was not possible to standardize the quantity of water used to prepare a dough of certain weight because of the changes in consistency of the dough effected by fermentation. The dough thus mixed was removed from the mixer and spread out in a sheet about 2 cm. thick on a generously oiled glass plate. The surface of the dough was also oiled—a colorless paraffin oil being used for the purpose—and allowed to stand for about 20 minutes. At the end of this time discs of dough about 50 cm. in diameter were cut out with a cork borer and the extensibility was measured in the way described. Ten

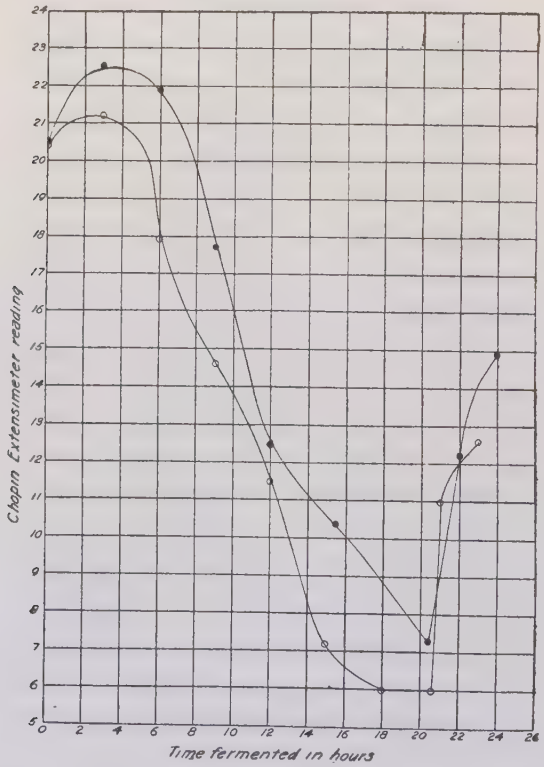

Fig. 13. Changes in Extensibility in Two Sets of Observations

The readings up to the 20th hour indicate the changes resulting from the fermentation of the sponge; those after the 20th hour represent changes coincident with the preparation of the dough and subsequent to the mixing of the dough.

to twelve determinations of the extensibility were made and the value given in the table is the mean of these determinations.

Doughs were prepared for these tests at 3-hour intervals. In Table XVII and Figure 13 are given the results which were obtained. In Figure 13 the time in hours is plotted on the axis of abscissa and the square root of the volume of the bubble as read on the measuring cylinder N of the Chopin apparatus is plotted on the axis of ordinates. These data show that during the first 3 to 6 hours the extensibility of the dough actually increased. This was due no doubt to a more complete hydration of the proteins which occurred during this time. After complete hydration of the protein was attained, however, the extensibility fell at a rapid rate. In the dough represented by open circles in Figure 13, the extensibility fell from 21 to 6. The value 6 is not a constant low level as indicated by the graph. This value is probably the lowest limit that the instrument is capable of measuring. It required a pressure equal to a reading of 6 on the extensimeter scale to raise the thin sheet of dough from the metal surface of the plate. These data are substantiated by the results of viscosity determinations discussed in the following section. At the end of the 18-hour fermentation period, or when the additional flour and the sodium bicarbonate are added, the extensibility increased. When two sponges were compared in which there was a substantial difference in extensibility, the same order of extensibility was found in the resultant doughs after remixing with the additional flour and the soda, just as might be anticipated.

TABLE XVII

EXTENSIBILITY OF CRACKER DOUGH DURING PROGRESSIVE STAGES OF FERMENTATION AS DETERMINED BY THE CHOPIN EXTENSIMETER

Dough No. 1			Dough No. 2		
Time Hrs.	Treatment	Extensibility	Time Hrs.	Treatment	Extensibility
0	Fermentation	20.4	0	Fermentation	20.5
3	"	21.2	3	"	22.5
6	"	17.9	6	"	21.9
9	"	14.6	9	"	17.7
12	"	11.5	12	"	12.5
15	"	7.2	15.5	"	10.4
18	"	6.0	20.5	"	7.3
21	"	6.0	22	Addition of flour and $NaHCO_3$	12.2
22	Addition of flour and $NaHCO_3$	9.2	24	Fermentation	14.9
26	Fermentation	12.6			

During this long fermentation period, notable changes were evident in the dough membrane which was formed when the sheet of dough was stretched in the Chopin apparatus. Originally this membrane was as fine and thin as tissue paper and the bubbles were almost phenomenal in size. As fermentation progressed, the bubbles blown

from the dough became smaller and the walls of the bubbles thicker. Finally at the 18-hour stage an even membrane did not form at all. The surface of the dough was roughened and reticulated with uneven clusters of dough extending at right angles to the strands.

The fact that the extensibility increased when additional flour and the sodium bicarbonate were added, raised a question concerning the mechanism of the change. To determine whether it was entirely due to the added flour or to changing the reaction of the dough to approximately the isoelectric point of the proteins, a series of experiments was conducted. A fermented dough of hydrogen-ion concentration corresponding to a pH of 4.45 was found to have an extensibility of 7.2. When bicarbonate was added to separate portions of the dough in such quantities as to make the hydrogen-ion concentration equivalent to pH values of 6.31 and 7.01, slight increases in extensibility to 8.8 and 7.9, respectively, were noted. Upon the addition of flour and bicarbonate the extensibility increased to 11.9. Another dough of pH 4.38 showed an extensibility of 7.9. Upon the addition of bicarbonate to two portions to bring the pH to 6.07 and 7.05, the extensibility in both cases increased to 8.6. When flour alone was added the extensibility increased to 12.7, and when flour and bicarbonate were added the extensibility attained the same value. These data indicate that the increase in extensibility of the dough at the time when flour and sodium bicarbonate were added was due largely to the effect of the unaltered proteins of the added flour. The differences in extensibility between dough and dough plus sodium bicarbonate are probably within experimental error for the apparatus.

It is interesting to note the subsequent increase in extensibility of the dough to which fresh flour had been added. This is no doubt due to more perfect hydration of the proteins.

From these data it is evident that certain changes in the proteins have occurred which are not reversible by change of hydrogen-ion concentration. The next step is, obviously, to determine whether these changes can be induced by hydrogen-ion concentration changes alone. A fresh dough was accordingly brought to a pH of 3.63 by the addition of lactic acid and its extensibility determined at once. The extensibility was found to be 15.5 as compared with 20.9 for the normal fresh dough. A portion of the acid dough was then brought to pH 7.10 with sodium bicarbonate and its extensibility determined. It showed an extensibility of 15.9. Twenty-four hours later the extensibilities of the normal dough and the acid dough of pH 3.63 were again determined. The normal dough showed an extensibility of 23.0, again an increase due to more perfect hydration of the proteins, while that of the acid dough remained approximately constant at 15.4.

These experiments lead to the conclusion that the addition of acid reduced the extensibility of the dough and this effect was irreversible as neutralization of the acid failed to restore the original extensibility. The hydrogen-ion concentration, once having exerted its effect, ceased to function and hence the extensibility of such a yeast-free acid dough was constant for 24 hours at least. Altho the hydrogen-ion concentration in this acid dough was greater than is usually attained in doughs rendered acid by fermentation, the decrease in extensibility was less than results from fermentation with yeast. This suggests that the major changes in extensibility and physical properties of the gluten were not effected by the development of acidity but by a proteolysis. No attempt was made definitely to determine whether the agents of proteolysis are proteases of flour, proteases from ruptured yeast cells, micro-organisms present in the flour and yeast, or conditions induced in the fermenting dough by all these and other considerations.

The work of Sharp, Gortner, and Johnson (1923) showed that the treatment of a flour with alcohol had deleterious effects on the gluten as noted in bread-making experiments. These observations suggested that perhaps the alcohol developed in the fermentation process might exert such effects on the gluten of the cracker dough. Doughs were prepared with 2 and 5 per cent aqueous alcohol solutions and the extensibilities determined. The extensibility of the dough prepared with 2 per cent alcohol was 21.2 when fresh as compared with the control of 22.0, and had increased to 22.5 at the end of 24 hours as compared with 23 for the control at this time. This indicates no deleterious effects from the alcoholic treatment at this concentration, the same tendency for greater extensibility of the dough at the end of 24 hours due to hydration being noted. The dough prepared with 5 per cent alcohol, which is greater than the concentration of alcohol in the dough at the end of the fermentation period, showed an extensibility of 20.0 when mixed and of 15.4 at the end of 24 hours. Thus it is likely that the quantity of alcohol formed in the dough does not operate so as to affect the properties of the proteins to the extent indicated in the studies on the extensibilities of fermented doughs.

Effect of prolonged fermentation on viscosity.—Olsen (1923) followed the effects of yeast activity on a flour suspension by means of viscometric measurements. It was suggested that such methods would indicate in another and perhaps more exact manner, the changes which were taking place in the proteins of a fermenting dough. Several series of determinations of the viscosity of cracker sponge suspensions were accordingly made, using a Wallace & Tiernan viscosimeter.

In preparing the suspensions from the acid doughs to be used in these viscometric studies, it was found necessary to bring the reaction

of the solution to the isoelectric point of the glutenin, as the latter in acid solution had imbibed so much water and was dispersed to such an extent that it was impossible to remove it from suspension by gravimetric methods. In order to precipitate the proteins, dilute sodium hydroxide was added until flocculation took place. As electrolytes had been added it was considered necessary to wash such precipitated proteins until the electrolytes were removed in order to produce the maximum viscosity of the suspension. Preliminary experimentation indicated that four successive washings with one liter of water each were required. After the last decantation, the precipitate was made to 125 cc., placed in the viscosimeter bowl, acidified, and the viscosity determined in the usual manner. Thirty-five grams of dough, corresponding to about 24.6 grams of flour, suspended in 125 cc. of water, yielded preparations the viscosities of which were within the limits of least experimental error of the viscometric method throughout the fermentation period. The precipitated protein suspension was brought to a volume of 125 cc., as in certain cases this was the least volume from which the wash water could be decanted without loss of solid materials.

Figure 14 presents in graphic form the data which were obtained during the 18-hour fermentation, and a final determination made at the end of 48 hours on the same dough. Determinations were not

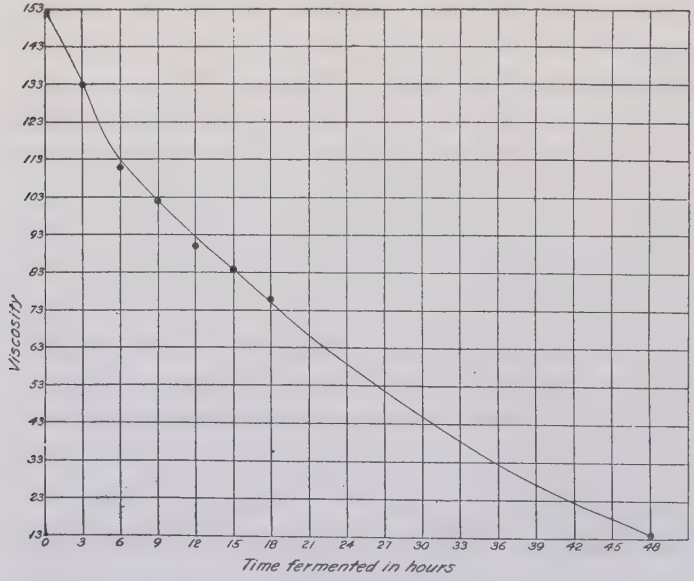

Fig. 14. Changes in Viscosity of Acidulated Sponge Suspensions as They Occur During Extended Fermentation Period in Cracker Sponge

The fermentation was allowed to proceed for more than twice as long as is usually allowed in fermenting a cracker sponge.

made on the dough after the addition of flour, sodium bicarbonate, and sodium chloride, as the results can be predicated and there would be difficulty in removing completely the added electrolytes.

A study of Figure 14 shows the regular decrease of viscosity of a washed and acidified dough suspension during progressive stages of fermentation. It is possible to follow the changes in properties of the proteins for a longer period through the use of the viscometric method than with the Chopin extensimeter, as with the latter the readings reach a constant value before changes in these properties cease. These data, however, serve to confirm and amplify the conclusions already reached as a result of observations made with the Chopin apparatus.

In the preparation of a sample of dough for the viscosity determination vigorous shaking is required, and some alteration of the properties of the proteins and consequent lowering of viscosity might be expected from this treatment alone. Experimentation demonstrated this to be a fact. Twenty-five grams of the original flour was extracted 4 times, using a liter of water for each extraction and the viscosity was determined in the usual manner. The viscosity of the flour was found to be 179 Wallace & Tiernan units as compared with 152 units for the fresh dough suspension. This would indicate that the treatment to which the dough had been subjected in mixing and subsequent suspension in water had caused a change in properties of the proteins equivalent to 27 units. It was also suggested that the shortening, the only substance besides the yeast present in the sponge, might exert a measurable effect, but comparative viscosity determinations indicated that no such effect occurred. The viscosity of the flour-water suspension to which the proportional quantity of shortening had been added was 182 units as compared with 179 units for the original flour.

The effect of acidity alone on the viscosity was determined. A flour-water suspension containing 24 grams of flour was brought to a pH of 4.08 with lactic acid. This acid suspension was allowed to stand for one hour and then the proteins were brought to the isoelectric point with sodium hydroxide solution. The precipitated protein was washed 4 times with distilled water in the manner previously described and the viscosity determined in the usual way. The viscosity of this suspension was 210 units as compared with 204 units for the once acidulated suspension. This appears to supply the additional evidence that acids of fermentation are incapable of accomplishing by themselves the profound changes in the physical properties of the protein which are found to occur during fermentation.

In order to investigate the effect on viscosity of any salts which might be formed in the process of bringing the proteins back to the isoelectric point, 2 grams of sodium acetate was deliberately added to a flour suspension. The precipitated proteins were washed four times and the viscosity was determined. The viscosity was found to be 201 units as compared with 204 units for the original flour suspension. This indicates that the 4 washings of the flocculated proteins removed the salts to the extent at least that the salt effect is the same under standard conditions. The last experiment shows that the lowering of viscosity is due to actual degradation of maximum water imbibing capacity of the proteins and not to salt effects which tend to prevent imbibition. This suggests that a modification of the properties of glutenin may have occurred which would be evidenced by a change in the "quality constant" or constant *b*, as the term was applied by Gortner (1924). One hundred grams of dough which had been allowed to ferment for 48 hours was shaken with distilled water until suspension was complete. Aliquots of this suspension equivalent to 17.57, 24.60, and 31.63 grams of flour were taken, the proteins brought to the isoelectric point, washed 4 times with distilled water, and the viscosities determined in the usual manner. These viscosities were found to be 6.75, 14.00, and 26.00 units respectively for the three flour concentrations prepared as stated above. From these data the quality constant *b* was calculated and found to be 2.27. The constant *b* of the original flour determined in the same manner was found to be 3.10. A comparison of these quality constants indicates that the fermentation has exerted a deleterious effect on the quality of the glutenin of the flour. Here again it is not possible to explain the mechanism of the change. It may be brought about by a cleavage of the protein molecule into two or more large segments, and in view of the fact that products of more complete hydrolysis of the proteins make their appearance during fermentation, the opinion is expressed that this is what actually occurs. On the other hand the change may be a purely physical one brought about by changes in aggregation of the particles during the fermentation process.

From all angles from which studies of the proteins and their changes during fermentation have been viewed, it appears that physical properties—elasticity, tenacity, and viscosity—are impaired during the processes of fermentation.

Diastatic activity in cracker doughs.—Sugars are not commonly added to cracker sponges, and the gases of fermentation must accordingly be produced in large part from sugars, which in turn are produced from starch in consequence of the activity of the diastatic enzymes of the dough. The presence of diastase must be attributed largely

to the flour, as normal, vegetative yeast cells do not secrete an active diastase. Rumsey (1922) has shown that flours vary widely in diastatic activity. It appeared desirable to determine the relative diastatic activity of representative cracker flours, and for this purpose five of the flours referred to in Table III were selected. Rumsey's (1922) methods were used except that the sugar content was determined by the method of Quisumbing and Thomas (1921). While this phase of the study merits further attention, the data presented in Table XVIII indicate clearly that considerable variation in this important property of cracker flours will be encountered. Failure of certain cracker sponges to rise properly in the trough may perhaps be due to a low diastatic activity of the flour, such as is exhibited by Flour No. 5. Flour No. 3, on the other hand, shows an unusually high diastatic activity, and, judged from this standpoint alone, might be useful in blending with other flours deficient in this particular.

TABLE XVIII

COMPARATIVE MEASUREMENTS OF DIASTATIC ACTIVITY OF FIVE SAMPLES OF FLOUR

Flour sample No.	Reducing sugar as maltose per 2.5 gm. flour	Reducing sugar as maltose after 1 hr. digestion per 2.5 gm. flour	Diastatic activity, maltose per 10 gm. flour
	mg.	mg.	mg.
1	16	65	196
2	13	67	216
3	11	115	416
4	8	45	148
5	8	37	116

The quantity of sugar present in a cracker sponge at any particular time is affected by the joint operation of two independent phenomena. In the first of these, sugar is produced in consequence of the activity of the flour diastase. In the second, sugar is converted into carbon dioxide and alcohol in consequence of fermentation which results from the presence of yeast. To ascertain how these affected the sugar level of the sponge, an experiment was conducted in which the concentration of sugar was determined at 3-hour intervals for 21 hours, and again at the end of 48 hours. The flour used contained .60 per cent of reducing sugar (as maltose) and the diastatic activity was 216 in terms of Rumsey units. From the resulting data, recorded in Table XIX, and graphed in Figure 15, it appeared that during the first 6 hours after mixing, the dough accumulated sugar in consequence of the low rate of fermentation. The increase in sugar content was less than might be anticipated from the measurement of diastatic activity. Swanson and Calvin (1913) have shown, however, that in a concentrated flour-water mixture diastatic activity was at a lower level than in the less concentrated mixtures such as are used in the Rumsey method.

After 9 hours the sponge was fermenting at an accelerated rate, and thereafter the sugar disappeared rapidly, reaching a very low level at the end of the 21st hour. As this is about the usual period of cracker sponge fermentation, it may accordingly be concluded that there is little or no sugar present at the time the sponge is remixed into dough.

When the cracker sponge remained undisturbed for 48 hours, the sugar content reached a higher level than at the end of the initial 21-hour interval. This was probably the result of yeast activity being inhibited by the relatively high hydrogen-ion concentration (pH 3.8), or through the accumulation of some other product of yeast metabolism.

TABLE XIX

REDUCING SUGAR CONTENT PER 5 GRAMS OF CRACKER DOUGH DETERMINED AT 3-HOUR INTERVALS DURING PROGRESSIVE STAGES OF FERMENTATION

Time hrs.	Maltose per 5 grams of cracker dough mgs.
0	42.5
3	46
6	50
9	43
12	21
15	15
18	12
21	6
48	30

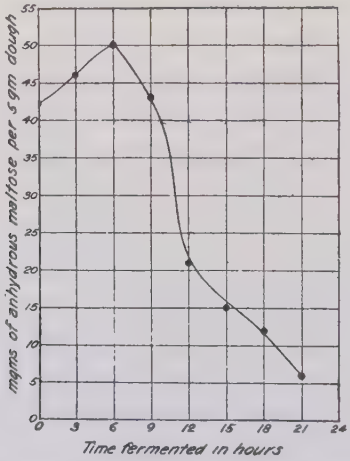

Fig. 15. Reducing Sugar Content (Calculated as Anhydrous Maltose) of a Cracker Sponge at Various Stages During Fermentation

In another study of the sugar content of fermenting cracker sponge and dough, 5 grams of the sponge, just before remixing, contained 12 milligrams of maltose, while 3 hours after the addition of the bicarbonate and remixing, the same quantity of dough contained 14 milligrams of maltose, or practically the same as the fermented sponge.

Losses in a cracker dough due to fermentation.—Diastatic activity and the resulting production of fermentable sugars in a cracker dough are largely responsible for the total quantity of sugar which is fermented to carbon dioxide and alcohol. Each unit of sugar fermented represents a unit loss of dry matter, and the determination of this loss is of practical importance. In determining the actual loss of dry matter, as indicated by the production of carbon dioxide, the fermentation is assumed to follow the reaction:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

Thus each unit of carbon dioxide produced in fermentation would represent 2.047 units of sugar fermented, and hence of dry matter loss. This carbon dioxide is the sum of (1) that dissolved in the dough solution, (2) that lost from the dough in the gaseous form, and (3) that retained in a gaseous form in the dough and causing an increase in the volume of the latter. For determining the quantity of (2) and (3) the device described by Bailey and Johnson (1924) was used. A portion of dough representing 80 grams was placed in the device, and the quantity of gaseous carbon dioxide present in and escaping from the dough was determined. As shown by the data in Table XX and graphed in Figure 16, the quantity of carbon dioxide thus encountered during the first six hours was relatively small. Expansion of the dough began actively thereafter, and the volume of the dough reached its maximum after about eleven hours. Toward the close of the 19-hour period, hydrolysis of the gluten reduced the ability of the sponge to retain the gases of fermentation and the dough began to recede. After the eleventh hour most of the gaseous carbon dioxide escaped from the dough as fast as produced; in fact

TABLE XX

INCREASE IN VOLUME OF DOUGH, VOLUME OF CO_2 LOST FROM DOUGH, AND SUM OF THESE TWO VOLUMES AS DETERMINED AT 1-HOUR INTERVALS

Time	Increase in volume of dough	Increase in volume of dough and CO_2 lost from the dough	Volume of CO_2 lost from the dough
hrs	cc.	cc.	cc.
0	0	0	0
6	14	14	0
7	22	29	7
8	43	54	11
9	52	80	28
10	56	107	51
11	69	172	103
12	72	215	143
13	73	280	207
14	74	349	275
15	74	407	333
16	74	460	386
17	73	511	438
18	73	568	485
19	72	617	545

about 75 per cent of the total carbon dioxide produced in the fermenting sponge escaped in the gaseous form. The total gaseous carbon dioxide accounted for was calculated to weigh 1.067 grams. That dissolved in the dough solution was determined, and found to be 0.192 grams. Total carbon dioxide formed was accordingly 1.259 grams per 80 grams of sponge, equivalent to 2.580 grams of glucose. Recalculating to the basis of a 1200-pound sponge, this would be equivalent to 39.05 pounds of glucose. As the flour contributed 5.21 pounds of sugar to this dough, the sugar formed in consequence of diastatic activity was 33.84 pounds. In terms of starch converted into glucose, this would be equivalent to 30.46 pounds. The total loss in dry weight of the original constituents accordingly amounted to 35.67 pounds, which is equivalent to 3.5 per cent of the flour used in the dough.

The calculations thus far have considered only the loss up to the time the sodium bicarbonate is added to the dough. Fermentation also goes on after the addition of the bicarbonate but it is probable

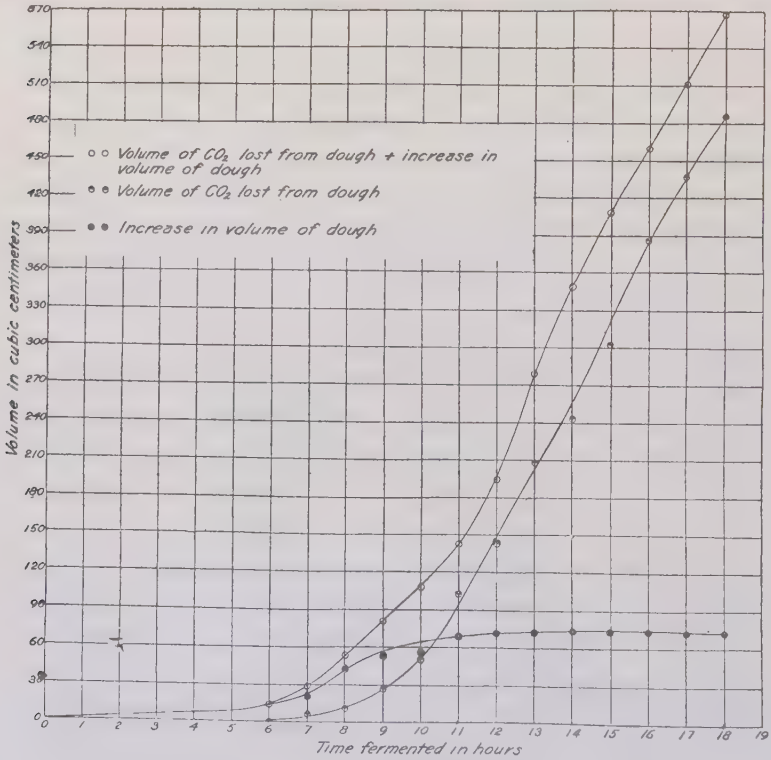
Volume of CO_2 lost from dough + increase in volume of dough
Volume of CO_2 lost from dough
Increase in volume of dough
Volume in cubic centimeters
Time fermented in hours

Fig. 16. Graph Showing (a) Volume of Carbon Dioxide Lost from a Cracker Dough, (b) Increase in Volume as Measured by Use of the Apparatus Devised by Bailey and Johnson (1924)

that most of the carbon dioxide formed at this period is retained in the dough, fresh flour having been added and the gluten consequently rendered more capable of retaining the gas. Assuming that no carbon dioxide is lost from the dough and that the dough itself occupies one-half the volume of the fermentation trough but completely fills the same trough before being baked into crackers, a calculation indicates that about 5 pounds of glucose will be fermented in producing the carbon dioxide required to effect this increase in volume. This means that after the addition of the bicarbonate, fermentation results in the further loss of one-half of one per cent of the dry weight of flour which was a part of the dough. Hence the total loss of dry matter in a process of fermentation such as this is 40.67 pounds per 5-barrel dough, or approximately 4.0 per cent of the flour.

Other processes of cracker manufacture.—The experimental work which has been reported thus far has been confined to doughs prepared according to the formula given. The more general practice in the industry, however, is to prepare a sponge which contains considerably less of the flour than is used in the straight dough process. Such sponges are usually prepared in such a manner as to contain the total quantity of water which is present in the finished dough together with proportions of flour which vary widely. The "slackest" sponge which has come to our attention was prepared by an eastern biscuit manufacturer. In this case 300 pounds of flour and 250 pounds of water were mixed into a sponge and allowed to ferment for an 18-hour period. At the end of this time additional flour and other ingredients were added.

A few experiments were conducted on soft sponges containing the total quantity of water (288 pounds) and 3 barrels of flour (588 pounds). The work was limited, however, to a study of rate of change of hydrogen-ion concentration as compared with the rate of such change in the type of sponge which had been studied more in detail. As was to be expected, the softer type of sponge increased in acidity the more rapidly. At the end of the 15-hour fermentation period, however, the rate of acid production in terms of both hydrogen-ion concentration and titratable acidity decreased in the softer sponge, while in the stiff sponge acid production was proceeding at a more rapid rate after the 15th hour. Hence at the end of the fermentation period it would not be impossible to have both soft and stiff sponges of the same hydrogen-ion concentration and titratable acidity. Such cases were actually encountered. A small portion of one of the soft sponges was allowed to ferment for 4 days. At the end of this time the pH had decreased only to 3.88, which is not as great a hydrogen-ion concentration as was found in the case of No. 5, a stiff sponge, shown in Table V. The

titratable acidity at this time also showed no great increase over that found after 15 hours. Twenty-five cc. of dough suspension, equivalent to 3.2 grams of sponge, required 13.4 cc. of 0.02 normal sodium hydroxide to neutralize it, using phenolphthalein as an indicator. Three days earlier an equal quantity of the sponge had required 12.6 cc. of the standard alkali solution to neutralize it. Hence during this 3-day period, acid equivalent to only .8 cc. of 0.02 normal sodium hydroxide had been formed.

While experimental work to determine the losses of carbon dioxide, or the losses in solids consequent to this loss of carbon dioxide, were not carried out on the soft sponge type of fermentation, it is believed that, in view of the greater rate of enzyme activity in such softer sponges, the percentage losses will be greater than in the stiff sponge. There is, however, less solid material in the soft sponge, hence the total loss in weight of solids from the two types of sponge (considering the two processes as a whole) per unit of flour in the finished batch may be about the same. No experimental evidence is available on this subject, altho the opinion has been expressed that a higher yield of crackers is obtained by use of the soft sponge process.

Attempts to increase the yield of crackers.—In view of the considerable losses in dry matter which occur during fermentation, a method is sought for bringing the dough into the proper condition for cracker baking without at the same time occasioning the loss of a large quantity of solid material. Several such methods have recently been proposed, one of which is used in several cracker bakeries. Both of these methods recommend the use of greater quantities of yeast. In one of these methods, one pound of yeast per barrel of flour is recommended. The sponge is set at a lower temperature than is the usual practice. It is claimed that at this lower temperature the desired changes in the properties of the dough are effected without as great loss in solid material as occurs at the higher temperature with less yeast. It has been claimed that in practice this method of fermentation increased the yield, but no data are available to the author to substantiate this statement. Some experimental work was done on a modification of this process in which slightly less yeast and a higher temperature were used than called for by the original method. The sponges at the end of the 18-hour fermentation period were decidedly less acid than those prepared according to the other processes. The greater number of such doughs had a pH which varied between 4.50 and 4.70 and consequently required on the average less sodium bicarbonate for neutralization than did sponges prepared according to the older methods. The quantity of sodium bicarbonate to be added to such sponges as these was determined in the same manner as was described in a pre-

ceding section. The crackers or their properties differed in no essential particular from crackers produced from doughs fermented according to the more common methods.

The most radical departure from the older practices of fermentation and preparation of the dough for cracker manufacture was suggested by Blank. Opportunity was offered for a careful study of this process and the observations which were made are detailed. A large quantity of yeast was used, as well as a malt extract of high sugar content but with diastases inactivated by heat. A dough for a 6-barrel run of crackers was mixed and contained the following ingredients:

	Pounds
Flour	1036
Lard	79
Yeast	24
Water	353
Malt	15
Acetic acid (10%)	3.75

These were mixed for three minutes and the following then added:

	Pounds
Flour	143
Salt	18
Sodium bicarbonate	7

and the mixing continued for ten minutes. This dough, weighing 1678 pounds, was allowed to ferment for 5 hours, and then baked into crackers. During the fermentation period the dough decreased 4 pounds in weight. Assuming that this loss was due entirely to carbon dioxide which escaped from the dough, approximately 8 pounds of sugar was fermented. The actual loss of dry matter due to fermentation was probably less than this, as some moisture was certainly lost from the dough. As the dough completely filled the fermentation trough a certain quantity of gas was, however, weighed in the dough. It has been shown that 5 pounds of solid material would furnish enough carbon dioxide to cause the dough to fill the fermentation trough. In addition an appreciable quantity of carbon dioxide was dissolved in the dough solution. Basing our calculations on the quantity of carbon dioxide which had been found in a sponge solution, the carbon dioxide content of which had been determined (i. e., 192 mg. per 80 gm. of sponge), it would require 8 pounds of sugar to supply this carbon dioxide. The total loss to be expected would therefore be 21 pounds per 6-barrel dough, or 3.50 pounds per barrel of flour, equivalent to 1.78 per cent of the flour.

The quantities of materials on the moisture-free basis which might be considered as constituting a one-barrel cracker dough are as follows:

	Pounds
Flour	172
Shortening	14
NaCl	3.5
$NaHCO_3$	1.3
Yeast	1.0
Malt	1.2
Total	193.0

The loss of dry material on this dough would therefore be 3.50 divided by 193 or 1.81 per cent. Hence the loss of dry material was reduced to a considerable extent by the use of this method as contrasted with the usual long-fermentation procedure.

Assuming that the crackers contained 5 per cent of moisture, 193 — 3.50 pounds of dry material should yield 199.5 pounds of crackers. Actually 195.33 pounds of crackers were obtained. This dough, however, was baked into oyster crackers, and there is reason to believe some of the shortening may be lost in baking this type of crackers. A portion of the shortening in the cracker melts and runs out on the plate of the oven and is converted into acrolein and other compounds by the heat. This is supported by the observation of the men in charge of the ovens that the odors are more marked when crackers which do not cover the whole plate of the oven are baked.

Other doughs were prepared in the same manner as the one described and similar results were obtained. It is thus apparent that such a process does result in decreasing the losses of fermentation. The crackers produced by this process have properties, including flavor, somewhat different from those of ordinary crackers. Greater cost of ingredients used may offset the advantage of increased yield.

In the Blank process the addition of acetic acid is obviously intended to bring the dough into the same condition as results from the acids produced by fermentation. The acids produced during fermentation bring the dough to a hydrogen-ion concentration corresponding to a pH 4.0 $\pm$ .20, while the added acetic acid in the new process never made the dough more acid than an acidity corresponding to a pH 5.4. It has already been shown that the addition of acids alone does not effect the same changes in physical properties of dough as result from a prolonged fermentation period. The acid used in the Blank process probably has a slight effect on the dough and cracker properties aside from a possible contribution to the flavor of the latter. It may, on the other hand, serve as a means of immediately saturating or partially saturating the dough solution with carbon dioxide when the sodium bicarbonate is added. This gas may not be produced in sufficient quantity to effect this result in the relatively short fermentation period. The quantity of sodium bicarbonate to be used may be easily

calculated, however, and this is one of the outstanding advantages of the process, assuming that acid need be used at all.

The changes which occurred in the proteins during a fermentation process such as this were measured by means of a viscosimeter. A quantity of dough equivalent to 24 grams of flour was shaken until completely in suspension. The electrolytes were removed by 4 washings with distilled water, one liter of water being used for each washing. After the final decantation of water, the residue was made up to 125 cc. in a graduated cylinder and poured into the bowl of a Wallace & Tiernan viscosimeter. The liquid was brought to the desired level by means of suction, .5 cc. of 20 per cent lactic acid added, and the viscosity determined in the usual manner. In one instance the viscosity of the preparation from the special dough was 90 units, as compared with 26 units for the long fermentation process. In all cases in which comparisons were made the viscosity of the suspension of dough prepared by the Blank process was markedly higher than that prepared from the ordinary sponge. This indicates that the no-sponge-time process did not effect the same degradation in colloidal properties of the proteins as was effected by the long fermentation process.

Discussion

In each section of this paper as it has been developed, the experimental data have been discussed. No effort was made, however, to discuss the various sections in the light of each other or to draw conclusions concerning their significance in the whole process of fermentation. The discussion in the following paragraphs constitutes an effort to correlate the various parts of the experimental work which submit to correlation.

The typical flours used in the cracker industry are milled from soft wheats and contain on the average 9.28 per cent protein and .49 per cent ash. If flours high in protein are used, a higher percentage of shortening (which is expensive) must be added or the flours must be allowed to undergo a more extended fermentation in order to bring about the development of the proper "shortness" in the baked product. No matter what the protein content of the flour may be, fermentation is resorted to in order that the gluten may be "conditioned," or brought to the stage which gives the desired properties in the baked cracker. The changes, physical and chemical, which take place in the dough during the fermentation period have been the major problems in this research.

Most of the experimental work was confined to the stiff sponge type of fermentation process, in which flour constitutes about 72 per cent of the total weight. In such stiff sponges as this, it is inconceiv-

able that hydration of the flour may proceed rapidly to completion. By means of the Chopin extensimeter it was demonstrated that the extensibility increased during the first 6 hours after the sponge had been mixed. This was attributed to a progressive hydration of the flour constituents during this time.

Simultaneously with the hydration of the proteins an increase in reducing sugars in the dough was noted. This indicated that during this early period diastases were more active in producing sugars than the enzymes of fermentation were in converting them into carbon dioxide and alcohol. Determinations of increase in volume of the dough during this period showed that almost no gaseous carbon dioxide was formed. Hence it appeared that diastases are active while the yeast enzymes did not appear to function, in such a manner at least as to liberate gaseous carbon dioxide, altho some carbon dioxide was undoubtedly present in the dough solution. Acid-forming organisms were also extremely slow in rendering the dough acid during this early period.

The dough, once thoroly wetted, became an actively fermenting mass. Hydrogen-ion concentration increased at a rapid rate. Thus during a 2-hour period it increased frequently to 9 times the concentration at the beginning of that period. Yeast activity caused carbon dioxide to be formed and this in turn inflated the dough, so that at the end of an 11-hour period it had its maximum volume. After 11 hours practically all the carbon dioxide formed was lost from the dough. It is assumed that by this time the dough solution had become saturated with gas. As rate of fermentation increased the quantity of reducing sugars present in the dough decreased. This does not mean that diastatic activity decreased. On the other hand, diastatic activity probably proceeded at a more rapid rate than during the earlier stage, as during the whole 18-hour process of fermentation about 20 pounds of starch must have been converted to fermentable sugars. The enzymes of fermentation, however, utilized the sugars faster than they were formed and the net result was a decrease in the content of reducing sugars in the dough.

Coincidently with these enzymic phenomena, certain changes occurred in the properties of the proteins. When a thin sheet of sponge of unit area taken from the sponge at the end of a 12 to 15-hour fermentation period is extended by means of the Chopin apparatus, a value of 12 or less may be obtained as compared with twice that value for the original dough, or the dough at any time during which hydration alone is proceeding. The viscosity of acidulated suspensions prepared from the sponge at the end of this 12 to 15-hour fermentation period was considerably lower than at any previous period,

indicating that a profound change had occurred in certain physicochemical properties of the glutenin, particularly the hydration capacity. Increases in nitrogen nonprecipitable with tin or copper were small, but indicated that a cleavage of the protein into small molecules was taking place. The protein in the aqueous solution of fermenting sponges, however, showed a remarkable increase during this period. This was, obviously, due to the effect of acids on the imbibitional capacity of the proteins. Thus before rapid acid production occurred, less than 20 per cent of the total protein of the flour was present in such centrifuged suspensions. Two hours later, owing to increase in hydrogen-ion concentration, the total protein in such aqueous solutions increased to 70 per cent.

Toward the end of the fermentation there was a period in which the activity of certain of the organisms was retarded, owing either to products of their own metabolism, or to products of metabolism of other organisms present in the fermenting mass. Among such reactions which showed a decrease in rate was that of acid formation. After 18 hours of fermentation only slight increases occurred in either hydrogen-ion concentration or titratable acidity. It is probable that the high acidity of the dough mass so operated as to retard the activity of those organisms or enzymes to which the development of acidity was due. If the fermentation is extended beyond 18 hours, there is some evidence that the enzymes of fermentation also tended to slow up in their activity. The evidence was indirect, however, and no effort was made to confirm it experimentally. It was based on the fact that at the end of a 48-hour fermentation period there was a distinct increase in reducing sugar present in the dough over that present after 21 hours of fermentation. This increase in reducing sugars occurred in spite of the fact that the dough had a hydrogen-ion concentration decidedly more acid than the optimum for the action of diastatic enzymes of flour.

During this later period of fermentation there appeared to be no tendency for proteolysis to be retarded. Even if fermentation was allowed to proceed for 24 hours beyond the usual time, proteolysis continued to take place. The nitrogen fractions nonprecipitable with tin and copper each doubled during the prolonged fermentation period. The viscosity of suspensions prepared from the fermented dough continued to decrease during prolonged fermentation. Thus, in a certain experiment the viscosity of an acidulated suspension of dough immediately after mixing was 152 Wallace & Tiernan units. At the end of 18 hours the viscosity had decreased to approximately one-half this value, and at the end of 48 hours it had further decreased to 14 units. The use of the Chopin extensimeter showed similar degra-

dation in physical properties of the proteins but it was not possible to follow the changes to the degree which viscometric methods permit.

No effort was made in this work to determine the exact mechanism of the proteolysis. Proteolysis may be only one of the factors which are of importance in effecting the proper "conditioning" of the cracker dough for cracker baking. It appears certain that this conditioning is a complex change and may be due to the operation of several factors. The acids and the alcohol produced during fermentation had little permanent effect on the physical properties of the proteins, so far as could be determined by the methods used in the experimental work. The acids caused the proteins to imbibe water and to disperse in solution, but this effect was apparently rendered nil by addition of sodium bicarbonate. It appeared that proteolytic enzymes were the agents which effected the greater part of the changes that occurred in conditioning the dough.

At the end of a fermentation period which was usually about 18 hours, sodium bicarbonate, sodium chloride, and the additional shortening and flour were added. The sodium bicarbonate neutralized the acids produced during fermentation. It was found that a slight excess of bicarbonate over that required was desirable. This excess of bicarbonate produced crackers of the proper color and lightness. When the added bicarbonate rendered the dough slightly alkaline (pH 7.10), crackers of the proper qualities for this particular region were produced. As has been stated, variations exist in the standards or criteria of properties which make for ideal crackers.

In addition to the effect produced by the sodium bicarbonate, the flour added in remixing the sponge exerted a certain effect on the properties of the dough. This fresh flour supplied a normal unaltered gluten which when added to the fermented dough increased the elasticity and tenacity of the latter. This increase in elasticity and tenacity as determined by the Chopin extensimeter was due entirely to the added flour and not to the fact that the proteins have been brought to approximately the isoelectric point.

The dough proper was allowed to ferment for 2½ to 4 hours longer and was then baked into crackers. If the proper excess of sodium bicarbonate was present in the dough, the increase in hydrogen-ion concentration during this fermentation was relatively slow. If insufficient bicarbonate was present the hydrogen-ion concentration increased at a rapid rate. Acid crackers were regarded as undesirable for the several reasons which have been given. When the proper excess of bicarbonate was present, a light cracker of desirable color was obtained.

Crackers obtained from slightly alkaline doughs were more alkaline than the dough from which they were baked for at least two reasons. In the first place most of the carbon dioxide present in the dough, which increased its hydrogen-ion concentration, was volatilized by the baking process. In the second place, the sodium bicarbonate present in the dough was converted into carbonate by heating. It was to this conversion of bicarbonate into the more alkaline carbonate that the greater part of the increase in degree of alkalinity which resulted during baking was due. The ideal cracker as judged by the standards of the geographical region in which these studies were made, had a pH of 7.4 to 7.9. The doughs from which such crackers were baked had a hydrogen-ion concentration corresponding to pH 7.00 to 7.10 immediately after the addition of the sodium bicarbonate. During the fermentation period the pH could fall to 6.80 and good crackers be produced, but this was the lowest limit of pH, if crackers were desired with the properties which have been mentioned.

During the long fermentation period relatively large losses in dry matter were found to occur. Basing calculations on losses measured on a small quantity of dough, such losses were found to be about 4 per cent of the dry matter which went into the preparation of the dough. Experimental work is now in progress to modify the methods of fermentation so that these losses may be reduced.

The experimental work described has been confined to a determination of certain chemical and physical changes which occur in cracker dough fermentation as practiced in the industry. A method has been evolved for determining the proper quantity of sodium bicarbonate to be added to the cracker dough. The effects of the hydrogen-ion concentration on the properties of the cracker as controlled by the quantity of added sodium bicarbonate have been noted. In view of the fact, however, that during the fermentation process 80 per cent of the carbon dioxide formed by the fermentation escapes immediately into the atmosphere without accomplishing any useful result, the fermentation process appears to be a wasteful method of effecting the desired properties in the dough. No change in procedure has been suggested in consequence of the experimental work. Having determined some of the major changes effected by fermentation, however, the next step in such research will include a study of other methods by which the same changes may be produced with a simultaneous reduction of the losses which must result from any fermentation process. The question may well be asked, is it necessary that the dough be expanded with carbon dioxide during the period just before baking? The machines which roll the dough into the thin sheets certainly express any gaseous carbon dioxide which may be there. Hence distend-

ing the dough to its maximum capacity with carbon dioxide may be unnecessary.

The significant results which are accomplished by the prolonged fermentation period are (1) the dough solution becomes saturated or partially saturated with carbon dioxide gas, and (2) a degradation in tenacity, elasticity, and extensibility of the gluten proteins occurs. It is possible that both of these results can be effected by other and less wasteful means than by extended fermentation.

Summary and Conclusions

Degree of acidity or alkalinity of crackers collected from factories throughout the United States varied widely. The most alkaline cracker in the series of 60 samples had an alkalinity corresponding to a pH of 8.81. The most acid cracker in the series had an acidity corresponding to a pH of 5.71. The average hydrogen-ion concentration of all the samples corresponded to a pH of 7.84.

Storage of the crackers resulted in an increase in acidity amounting to a decrease in pH of 0.3 during 7 weeks.

Development of rancidity did not appear to be correlated with the degree of alkalinity or acidity of the cracker. A few isolated observations indicate that in cases of extremely alkaline crackers (pH 10) rancidity may develop more rapidly.

Flours used in the cracker industry are in most cases milled from soft wheats. The average ash content of 121 such flours was .49 per cent, while the average protein content was 9.28 per cent.

Quality of the protein was better throughout the whole series of cracker flours than has been generally found in the bread flours of higher protein content. The average quality constant b for 66 cracker flours was found to be 3.31. A constant b of 4.43 was reported for a flour of 9.63 per cent crude protein. It is believed that this is the highest constant b which has yet been observed and reported.

Acidity developed during the fermentation of a cracker sponge effected a change in hydrogen-ion concentration of the sponge (in terms of pH) from a pH of 5.85 to a pH of approximately 4.0 .20. The increase in acidity was slow during the first 6 to 8 hours of fermentation, but after this it increased rapidly until a hydrogen-ion concentration corresponding to a pH of $4.0 \pm .20$ was reached. When the hydrogen-ion concentration of the sponge was increased to a pH of approximately 4.0, no appreciable change in degree of acidity or in titratable acidity resulted from prolonging the fermentation for an additional 24 hours.

Addition of sodium bicarbonate to the acid sponge neutralized the acids of fermentation. An excess of bicarbonate over that required to neutralize these acids has been found desirable for the production

of satisfactory crackers. In order that ideal crackers be produced, the cracker dough should have a sufficient excess of sodium bicarbonate that an unaspirated suspension prepared from the dough has a hydrogen-ion concentration corresponding to a pH of 7.00 to 7.10. Doughs more alkaline than this produced crackers which were too brown and occupied too much volume per unit weight of material. Doughs more acid than this became too acid during the 2½ to 4 hours subsequent to the addition of the sodium bicarbonate, and acid crackers resulted. Such acid crackers were too pale in color and occupied too little volume per unit weight of material.

The baking process converted the sodium bicarbonate to sodium carbonate with the liberation of carbon dioxide gas and water. Hence baked crackers were found to be more alkaline than the dough from which they were baked.

Caramelization of the sugars (browning of the cracker) takes place more readily in the presence of free alkali. This accounts for the "bloom" of crackers containing the proper excess of bicarbonate. Increased heating of alkaline crackers resulted in the production of weak organic acids which neutralized the alkaline reacting materials of the cracker, while increased heating of acid crackers had little effect on the hydrogen-ion concentration. The weak acids which result from caramelization appear to ionize so little as hardly to affect the hydrogen-ion concentration, yet they apparently react with the alkaline carbonate to reduce the alkalinity of the mixture.

Titration of a cracker sponge suspension to pH 8.4 with standard sodium hydroxide solution afforded a means of calculating the quantity of sodium bicarbonate required to neutralize the acids of fermentation. A similar titration of a flour suspension indicated the quantity of bicarbonate required to bring the added flour to the same hydrogen-ion concentration. Standard sodium bicarbonate solution could not be used for this titration, as at pH 7.00 to 7.10 increasing quantities of the solution had little effect on the hydrogen-ion concentration, hence it was not possible to obtain a sharp end point. An excess of sodium bicarbonate over that required to neutralize the acids of fermentation and of the raw flour was found to be necessary in remixing the doughs. In the case of the process studied at a certain biscuit factory, an excess of 1½ pounds of bicarbonate was found to give the results desired.

Hydration of the dough constituents proceeded progressively during the first 6 hours of the fermentation period. A degradation in such properties of the gluten as tenacity, viscosity, and elasticity began at the end of the 6-hour period and continued as long as the sponge was allowed to ferment. That this degradation was due partly, at least, to proteolytic cleavage of the proteins was indicated by the fact

that the nitrogen fraction nonprecipitable by tin or copper reagents increased in the sponge during the fermentation process.

Increased hydrogen-ion concentration which effected a marked increase in the solvation and dispersion of the gluten proteins was not the factor which operated to bring about the complete "conditioning" of the gluten.

Alcohol produced during the fermentation exerted no determinable effect in degrading the viscosity or elasticity of the proteins.

Reducing sugar content of the sponge increased during the first 6 hours of the fermentation process. After the 6-hour period the enzymes of fermentation functioned more rapidly than the diastases and the reducing sugar content of the sponge decreased. At the end of an abnormally extended 48-hour fermentation, however, the reducing sugar content of the sponge showed an increase over that at the end of 21 hours, which indicated that the acids or other products due to activity of the organisms present in the sponge tended to inhibit yeast activity more than they inhibited diastatic activity.

Carbon dioxide gas in these cracker sponges was not produced in sufficient quantity to effect an increase in volume of the sponge during the first 6 hours of fermentation. After the first 6 hours, and to the end of the fermentation period, carbon dioxide was evolved at a rapid rate. In the process studied, 10 per cent of the carbon dioxide formed was dissolved in the sponge solution. Another 10 per cent remained in the sponge and operated to increase the volume of the sponge, while 80 per cent escaped into the atmosphere.

Losses due to fermentation were calculated from the quantity of carbon dioxide produced during fermentation and it appeared that about 41 pounds of dry material per 5-barrel dough were converted into carbon dioxide and alcohol during a cracker fermentation process. This quantity was equivalent to about 4 per cent of the flour used in preparation of the dough.

The considerable losses due to fermentation have stimulated an effort to develop a method of effecting the desired changes in the dough and at the same time of reducing the fermentation losses. Thus far no method has been found which gives results which are uniformly satisfactory from the standpoint of the quality of crackers produced. The methods which have been suggested all involve the use of increased quantities of yeast, and some fermentation losses must accordingly occur. Further researches might be directed toward the development of a procedure which would effect an adequate degradation of the flour proteins through the use of suitable proteolytic enzymes and provide for the liberation of the necessary quantity of carbon dioxide in

the dough. The latter might be accomplished either in consequence of the reaction of bicarbonate with an acid, or by means of yeast fermentation, or both.

Literature Cited

Bailey, C. H.
1913. Relation of the composition of flour to baking quality. Canadian Miller and Cerealist, Vol. 5, pp. 208-209.

Bailey, C. H.
1920. A simple hydrogen electrode. Jour. Amer. Chem. Soc., Vol. 42, pp. 45-48.

Bailey, C. H. and Sherwood, R. C.
1923. The march of hydrogen-ion concentration in bread doughs. Jour. Ind. Eng. Chem., Vol. 15, pp. 624-627.

Bailey, C. H. and Le Vesconte, Amy.
1924. Physical tests of flour quality with the Chopin extensimeter. Cereal Chem., Vol. I, No. 1, pp. 38-63.

Bailey, C. H. and Johnson, A. H.
1924. Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. Cereal Chem., Vol. I, No. 6, pp. 293-304.

Baker, J. L. and Hulton, H. F. S.
1908. Some considerations affecting the strength of wheat flour. Jour. Soc. Chem. Ind., Vol. 27, pp. 368-376.

Blish, M. J.
1916. On the chemical constitution of the proteins of wheat flour and its relation to baking strength. Jour. Ind. Eng. Chem., Vol. 8, pp. 138-144.

Blish, M. J.
1918. A study of the non-protein nitrogen of wheat flour. Jour. Biol. Chem., Vol. 33, pp. 551-559.

Bovie, W. T.
1922. A new vessel for electrometric titration. Jour. Amer. Chem. Soc., Vol. 44, pp. 2892-2893.

Buchner, E. and Meisenheimer, J.
1909. Ueber die Rolle der Milchsäure bei der alkoholischen Gärung des Zuckers. Zeitsch. wiss. Landwirtschaft, 38, Ergänzungsband V. S. 265-288.

Buchner E. and Meisenheimer, J.
1910. Die chemischen Vorgänge bei der alkoholischen Gärung. Ber. d. deut. chem. Gesell., Bd. 43, S. 1773-1795.

Buscalioni and Fermi.
1898. Studio degli Enzymi proteolytici dei Vegetali. Annuario del R. Institute Botanico di Roma, Vol. VII, p. 99. (See Vines 1903.)

Chopin, M.
1921. Relations entre les properties mechaniques des pates de farines et la panification. Bul. Soc. Encour. Ind. Nat., t. 133, pp. 261-273.

Clark, W. M.
1922. The determination of hydrogen-ions. Williams and Wilkins Company, Baltimore.

Cohn, E. J. and Henderson, L. V.
1918. The physical chemistry of bread making. Science, Vol. 48, pp. 501-505.

Dean, A. L.
1905. On proteolytic enzymes. Bot. Gaz., Vol. 39, pp. 321-339.
Dean, A. L.
1905. On proteolytic enzymes. Bot. Gaz., Vol. 40, pp. 121-134.
Dernby, K. S.
1917. Studien über die proteolytischen Enzyme der Hefe and ihre Beziehung zu der Autolyse. Biochem. Zeitsch., Bd. 81, S. 107-208.
Effront, J.
1917. Biochemical catalysts in life and industry (See page 583). John Wiley and Sons, New York.
Fernbach, A. and Schoen, M.
1922. L'acide pyruvique dans la fermentation alcoolique. Compt. rend. soc. biol., t. 86, pp. 15-18.
Fernbach, A. and Schoen, M.
1923. Se forme-t-il de l'acide lactique dans la fermentation alcoolique? Compt. rend. soc. biol., t. 89, pp. 475-477.
Ford, J. S. and Guthrie, J. M.
1908. The amylolytic and proteolytic ferments of wheaten flours. Jour. Soc. Chem. Ind., Vol. 27, pp. 389-393.
Furth, O. and Sieben, F.
1922. Über Milchsauerzerstarung durch Hefe und durch Blutzellen. Biochem. Zeitsch., Bd. 128, S. 144-168.
Garup and Besanez.
1874. Über das Vorkommen eines diastatischen und peptonbildenen Fermentes in den Wickensamen. Sitzber de phys-med. Soc zu. Erlangen. (Original not seen.) See Vines (1906).
Garup and Besanez.
1875. Weitere Beobachtungen. Sitzber de phys-med. Soc. zu. Erlangen. (Original not seen.) See Vines (1906).
Geret, L. and Hahn, M.
1898a. Zum Nachweis des im Hefepressaft enthaltenen proteolytischen Enzyms. Ber. d. deut. chem. Gesell., Bd. 31, S. 202-205.
Geret, L. and Hahn, M.
1898b. Weitere Mitteilungen über das im Hefepressaft enthaltene proteolytische Enzym. Ber. d. deut. chem. Gesell., Bd. 31, S. 2335-2344.
Gortner, R. A.
1924. Viscosity as a measure of gluten quality. Cereal Chem., Vol. I, No. 2, pp. 75-81.
Hahn, M.
1898. Das proteolytische Enzym des Hefepressaftes. Ber. d. deut. chem. Gesell., Bd. 31, S. 200-201.
Hahn, M. and Geret, L.
1900. Über das Hefe-Endotrypsin. Zeitsch. f. Biol., Bd. 40, S. 117-173.
Hahn, M.
1903. Zur Kenntnis der reduzierenden Eigenschaften der Hefe. Die Zymasegärung, pp. 341-349. (Original not seen.) See Harden (1923).
Hansen, E. C.
1888. Undersolgelsen fra Gjaerings-industriens Praxis Meddeleser fra Carlsberg Laboratoriet. Bd. II, S. 257-322.

Harden, A. and Young, W. J.
1908. The alcoholic ferment of yeast juice. Proc. Roy. Soc. London, Series B., Vol. 80, pp. 299-311.
Jessen-Hansen, H.
1911. Etudes sur la farine de froment. Influence de la concentration en ions hydrogene sur la valeur boulangere de farine. Compt. rend. Lab. Carlsberg. t. 10, pp. 170-206. Translated in J. Amer. Assoc. Cereal Chem., Vol. 7, pp. 10-28.
Johnson, A. H.
1923. The relation of gas retention by wheat flour doughs to flour strength and fermentation. A thesis filed in the library of the University of Minnesota.
Koehler.
1892. Mitteilungen der Versuchstation f. Brau u. Maizerei. Wein, 1892.
Kostytschev, S.
1912. Ueber Alkoholgärung. Ueber die Bildung von Acetaldehyd bei der alkolholischen Zuckergärung. Zeitsch, physiol. Chem., Bd. 79, pp. 130-145.
Leighty, C. E. and Martin, J. H.
1922. The soft red winter wheats. U. S. Dept. of Agr. Farmers' Bulletin 1305.
Lindner.
1891. Die Mycoderma und die Praxis. Woch. f. Brau, Bd. 8, S. 667-669.
Mann, A. and Harlan, H. V.
1915. Morphology of the barley grain with reference to its enzyme secreting areas. U. S. Dept. of Agr. Bul. No. 183.
Methods of Analysis of the American Association of Cereal Chemists.
1919. The determination of nitrogen by the Kjeldahl method.
Michaelis, L.
1911. Die Bestimmung der Wasserstoffionkonzentration durch Gasketten. Abderhalden's Handbuch der Biochemischen Arbeitsmethoden, Vol. V, Part 1, pp. 500-524.
Nef, J. U.
1913. Dissoziationsvorgänge in der Zuckergruppe. Ann. d. Chemie, Bd. 404, S. 204-383.
Neuberg, C. and Hildesheimer.
1911. Ueber zuckerfreie Hefegärungen, I. Biochem. Zeitsch., Bd. 31, S. 170-176.
Neuberg, C. and Kerb, J.
1913. Ueber zuckerfreie Hefegärungen. XIII. Zur Frage der Aldehydbildung bei der Gärung von Hexosen sowie bei der sog. Selbstgärung. Biochem. Zeitsch., Bd. 58, S. 158-170.
Neuberg, C.
1922. Von der Chemie der Gärungs-Erscheinungen. Ber. d. deut. chem. Gesell., Bd. 55, S. 3624-3638.
Olsen, A. G.
1923. A study of the proteases of bread yeast S. cerevisiae. A thesis filed in the library of the University of Minnesota.
Olsen, J. C.
1913. Van Nostrand's Chemical Annual. D. Van Nostrand Co., New York.

Osborne, T. B.
1907. The proteins of the wheat kernel. Carnegie Institution of Washington, Pub. No. 84.
Osborne, T. B. and Leavenworth, C. S.
1916-17. Protein copper compounds. J. Biol. Chem., Vol. 28, pp. 109-123.
Quisumbing, F. A. and Thomas, A. W.
1921. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Elimination of certain errors involved in current methods. Jour. Amer. Chem. Soc., Vol. 43, pp. 1503-1525.
Rumsey, L. A.
1922. The diastatic enzymes of wheat flour and their relation to flour strength. Amer. Inst. of Baking, Bul. No. 8.
Scherning, H.
1897. Beitrage zur Chemie der Proteinfällungen. Mitsch. anal. Chem., Bd. 36, S. 643-663. The Analyst, Vol. 23, pp. 104-106. (1898)
Sharp, P. F. and MacDougall, F. H.
1922. A simple method of electrometric titration in acidimetry and alkalimetry. Jour. Amer. Chem. Soc., Vol. 44, pp. 1193-1196.
Sharp, P. F. and Gortner, R. A.
1923. Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. Minn. Agr. Exp. Sta., Res. Bul. 19.
Sharp, P. F., Gortner, R. A. and Johnson, A. H.
1923. Physico chemical properties of strong and weak flours. VII. The physical state of the gluten as influencing the loaf volume. Jour. Phys. Chem., Vol. 27, pp. 982-987.
Sharp, P. F. and Elmer, R.
1924. Wheat and flour studies I. Auto digestion of flour milled from frozen and non-frozen wheat harvested at various stages of maturity. Cereal Chem., Vol. I, No. 2, pp. 83-105.
Shaw, G. W. and Gaumnitz, A. J.
1911. California white wheats. Calif. Agr. Exp. Sta. Bul. No. 212.
Shollenberger, J. H. and Clark, J. A.
1924. Milling and baking experiments with American wheat varieties. U. S. Dept. of Agr., Bul. No. 1183.
Shutt, F. T.
1907. Quality in wheat. The relation of composition to bread making value. Canada Dept. of Agr. Bul. No. 57, pp. 37-51.
Snyder, H.
1899. The proteids of wheat flour. Minn. Agr. Exp. St. Bul. No. 63, pp. 520-530.
Sorensen, S. P. L.
1909. Enzymstudien II. Biochem. Zeitsch., Bd. 21, S. 131-304 (vide p. 292).
Sorensen, S. P. L.
1912. Die Formoltitration. Handbuch der Biochemischen Arbeitsmethoden herausgegeben von E. Abderhalden, Bd. VI, S. 262-277.
Stockham, W. L.
1920. Some factors related to the quality of wheat and strength of flour. No. Dak. Agr. Exp. Sta. Bul. No. 139.

Swanson, C. O. and Calvin, J. W.
1913. A preliminary study on the conditions which affect the activity amylolytic enzymes in wheat flour. Jour. Amer. Chem. Soc., Vol. 35, pp. 1635-1643.
Swanson, C. O. and Tague, E. L.
1916. A study of certain conditions which affect the activity of proteolytic enzymes in wheat flour. Jour. Amer. Chem. Soc., Vol. 38, pp. 1098-1109.
Thomas, L. M.
1917. A comparison of several classes of American wheats and a consideration of the factors influencing quality. U. S. Dept. of Agr. Bul. No. 557.
Truog, N.
1915. Method for the determination of carbon dioxide and a new form of absorption tower adapted to the titrimetric method. Jour. Ind. Eng. Chem., Vol. 7, pp. 1045-1049.
Vines, S. H.
1902. Tryptophane in proteolysis. Ann. Bot., Vol. 16, pp. 1-22.
Vines, S. H.
1903a. Proteolytic enzymes in plants I. Ann. Bot., Vol. 17, pp. 237-264.
Vines, S. H.
1903b. Proteolytic enzymes in plants II. Ann. Bot., Vol. 17, pp. 597-618.
Vines, S. H.
1904. Proteases in plants. Ann. Bot., Vol. 18, pp. 289-317.
Vines, S. H.
1905. Proteases in plants III. Ann Bot., Vol. 19, pp. 171-190.
Vines, S. H.
1906. The proteases in plants IV. Ann. Bot., Vol. 20, pp. 113-124.
Vines, S. H.
1908. Proteases in plants V. Ann. Bot., Vol. 22, pp. 103-121.
Vines, S. H.
1909. Proteases in plants VI. Ann. Bot., Vol. 23, pp. 1-18.
Wehmer, C.
1895. Über die Verflussigung der Gelatine durch Pilse Chemiker Zeit. No. 91, p. 2088. (Reviewed by Will, Cent. f. Bakt. u. Par. II Abt., Bd. 2, S. 92-96.—1896.)
Zinn, J.
1923. Correlations between various characters of wheat and flour as determined from published data, from chemical milling, and baking tests of a number of American wheats. Jour. Agr. Res., Vol. 23, pp. 529-548.

ACKNOWLEDGMENTS

This investigation was carried out under a fellowship grant from Mr. Albert P. Strietmann, of Cincinnati, Ohio, President of the Biscuit and Cracker Manufacturers' Association of America. Mr. Strietmann, moreover, provided additional funds which made possible the publication of this research. The author takes this opportunity to express his yratitude and appreciation to Mr. Strietmann for his active and enthusiastic interest in the work.

The research was conducted in the laboratories of the Sanitary Food Manufacturiny Company, of St. Paul; the Strietmann Biscuit Company, of Cincinnati, Ohio; and the Division of Agricultural Biochemistry of the University of Minnesota. The writer wishes to thank Mr. Leitch and Mr. Autrey, of the Sanitary Food Manufacturing Company, for their courtesy in allowing him to carry on experimental work under actual factory conditions.

To Dr. C. H. Bailey, under whose direction this research was conducted, the author is deeply indebted and takes this opportunity to express to him sincerest gratitude for the encouragement and assistance which he so freely gave. Without his kindly advice and inspiration the pursuit of this thesis would not have been possible.

The writer also wishes to thank Dr. R. A. Gortner for the many helpful suggestions which he gave; and Mr. A. V. H. Mory and Mr. A. A. Shaal for their interest in the work and for their aid in securing materials used in the experimental work.

Biography

Arnold Harvey Johnson was born in Minneapolis, Minnesota, November 21, 1896. His grade school education was obtained in the Minneapolis public schools. He was graduated from Central High School, Minneapolis, in 1916. In 1917 he entered the College of Agriculture, University of Minnesota, from which college he received the B. S. degree in 1921. From June, 1921, until the present time he has been registered in the Graduate School of the University of Minnesota. During the years 1921 and 1922 he held assistantships in Agricultural Biochemistry. During the summer quarter of 1922 he was a member of the research party which, under the direction of Dr. J. A. Harris, of the Carnegie Institute of Washington, investigated alkaline and drouth resistant vegetation in Utah and Arizona. In April, 1923, he was appointed Strietmann Fellow in Biochemistry, which fellowship he held until June 30, 1924.

In December, 1923, he received the M. S. degree in Agricultural Biochemistry with a minor in Organic Chemistry. His thesis subject was, "The Relation of Gas Retention by Wheat Flour Doughs to Flour Strength and Fermentation."

In May, 1924, he presented to the Graduate School of the University of Minnesota a thesis on, "A Physico-Chemical Study of Cracker Dough Fermentation."

He is a member of the following organizations:

The American Chemical Society
Alpha Zeta
Gamma Alpha
Sigma Xi
Phi Lambda Upsilon—President of the Minnesota Chapter for the year 1923-24.

Publications:

Studies on Wheat Flour Grades, III. Effect of chlorine bleaching upon the electrolytic resistance and hydrogen-ion concentration of water extracts. C. H. Bailey and A. H. Johnson. J. A. O. A. C., Vol. 6, pp. 63-68. 1922.

The physico-chemical properties of strong and weak flours, VII. The physical state of the gluten as influencing loaf volume. P. F. Sharp, R. A. Gortner, and A. H. Johnson. Jour. Phys. Chem., Vol. 27, pp. 942-947.

Studies on Wheat Flour Grades, IV. Changes in hydrogen-ion concentration and electrolytic resistance of water extracts of natural and chlorine treated flour in storage. C. H. Bailey and A. H. Johnson, Cereal Chem., Vol. I, No. 3, pp. 133-137.